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Contemporary Overview of Blood Concentrates in Oral and Maxillacial Surgery

Onur Gönül, Ahmet Usame Çiçek, Murat Afat, Onur Atali and Faysal Uğurlu

Abstract

It has always been a target to shorten and improve the healing process in medical field. Platelets with cytokines and growth factors in their structure have great importance on wound healing. Features of platelets gave the clinicians the idea of using platelet concentrates to promote the healing process. For this reason, many platelet-derived biomaterials have been tried in the medical field over the years. When approaching today, platelet concentrates have been found to be used medically, especially with the use of platelet rich plasmas (PRPs) and then platelet rich fibrins (PRFs). In particular, several studies conducted in recent years have revealed different blood concentrates. This chapter summarizes the development over time, properties and usage areas of blood concentrates in dentistry.

Keywords: platelet rich fibrin, platelet rich plasma, sinus lifting, growth factor, graft, regeneration, oral and maxillofacial surgery

1. Introduction

Platelets are the smallest, colorless blood cells which play a major role in coagulation cascade and prevent excessive blood loss. Platelets' diameters varies between 1-3 microns and they appear in a bright blue color under the microscope. Low platelet rate causes an increase in the tendency to bleed, and high platelet rate causes a clot (thrombosis) formation in the vein [1].

Platelets provide their functions with secretory granules in which there is a reservoir in its structure and freshly synthesized mediators. These granules consists ATP, ADP, serotonin, fibrinogen, plasminogen, platelet derived growth factor (PDGF), transforming growth factor beta (TGF- β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin like growth factor (IGF), etc. The growth factors have stimulating effect on wound healing by means of potential for matrix remodeling, cell proliferation and angiogenesis [2, 3].

According to several researches, main task of platelets in the body is hemostasis and final clot formation in bleeding areas. In addition to their hemostatic duties, they are also effective in the tissue repair process due to the long term effects of these growth factors in their structure [2, 3].

Platelets are counted among the potential cells of regenerative therapy. For the fact that they consist a large number of growth factors and are easy to obtain, has increased the interest in platelets. Tissue regeneration engineering is a field that many studies and attempts have been made about repairing a diseased tissue, regenerating or restoring a damaged tissue. Initially, studies have included allografts, xenografts, synthetic-based alloplasts, etc. However, most of these materials were observed to cause foreign body reaction in the body, which lead the studies to the human body's own tissues [4–7].

Ideas based on the use of human blood proteins as sources for regeneration arise from the idea that blood-derived proteins are a source of growth factors that can support angiogenesis and tissue growth for tissue regeneration. The healing process required for regeneration consists of hemostasis, inflammation, proliferation and maturation phases. Each phase requires its own specific proteins and cell types. However, since the non-human biomaterials used are avascular, the use of blood concentrates becomes more prominent [4–7].

As mentioned earlier, wound healing is a process that occurs as a result of hemostasis, inflammation, proliferation and maturation phases. Platelets are essential components that play an active role in the stages of hemostasis and fibrin formation, that is, in the early phases of the tissue regeneration process. Platelets secrete and contain some growth factors such as PDGF (Platelet derived growth factor), VEGF (Vascular endothelial growth factor), cytokines, etc., as well as angiogenetic factors that stimulate proliferation and activate the wound healing cells such as fibroblasts, neutrophils and mesenchymal stem cells [3, 8].

2. History of blood concentrates

The use of blood concentrates in medical field for various purposes is common for a long period of time. When we look at its historical background, the idea was used in the 1970s to be used for nerve tissue repair. The first use of platelet-rich plasma was reported in 1987 by Ferrari et al. during cardiac surgery and PRP was used to repair damaged cardiovascular tissues [9].

Looking at its use in dentistry, in the late 1990s, Whitman et al. suggested using PRP in oral surgical procedures, observing that PRP enhanced osteoprogenitor cells in bone graft and bone tissue. However, it is reported that depending on the use of bovine thrombin, coagulopathies and immune reactions can be observed [9, 10].

PRF is considered as the current platelet concentrate and has been used in oral and maxillofacial surgery since 2001. According to Choukroun et al. who first described PRFs in literature, PRFs have some advantages compared to PRPs, such as easier preparation and no chemical intervention to blood concentrates [11].

According to leukocyte and fibrin content, Verma et al. divided the platelet concentrates into 4 basic categories. They categorized platelet concentrates as P-PRP, L-PRP, P-PRF, and L-PRF [12].

Choukroun, the first person to report the use of PRF in 2001, described A-PRF, a new form of PRF, containing higher amounts of leukocytes in 2014. According to Choukroun, this new type of PRF has more potential than the original PRF [13].

3. Classification of blood concentrates

The story of blood concentrates is usually examined in 2 parts/generations. Here we see the PRPs first, and the PRFs as the second generation. Classification is simply done as follows:

- P-PRP (1. GENERATION)
- L-PRP (1. GENERATION)
- P-PRF (2. GENERATION)
- L-PRF (2. GENERATION)
- I-PRF (2. GENERATION)
- A-PRF (2. GENERATION)
- T-PRF (2 GENERATION)

4. Preparation procedures and clinical features of PRPs

Basically, the aim of the formation of blood concentrates is to achieve higher platelet density and high level of growth hormones. PRPs are also tried to be prepared for this purpose. PRPs are basically condensed plasma with a higher platelet concentration than the normal state of the blood. PRPs show high platelet and growth factor levels compared to normal blood. It has been determined that PRPs have positive effects on periodontal cells, osteoblasts, etc. after their use [9, 14–18].

Despite their benefits, PRPs have some disadvantages. They are obtained in a multi-stage and long process, difficult to handle and expensive to obtain. PRPs usually have two basic centrifuge steps. Ethylenediaminetetraacetic acid (EDTA) or citric acid can be used in the first slow centrifuge step. Then comes the second centrifuge step, which is applied faster. At this stage, additional substances such as calcium chloride or/and bovine thrombin can be used. These additives, in fact, cause blood concentrates, which are of interest as being autologous, to lose their full autologous feature. In addition, foreign substances -especially bovine thrombin- which are included in the PRPs, increase the potential of PRPs to create a foreign body reaction. Accordingly, the natural inflammatory process of the body can be disrupted. In addition, artificial clotting is provided while obtaining PRP. Accordingly, the fibrin matrix structure formed is different from the structure obtained naturally. The fibrin matrix structure obtained in this way is more rigid. Due to this rigid structure, it does not release the growth factors in the PRP in a controlled and long term and releases all at once. Also bovine thrombin can cause the risk of coagulopathy as well as foreign body reaction. It also increases the cost of preparing PRPs with special kits and spending a lot of time. PRPs are often used by mixing with grafts due to the disadvantage of their liquid structures during use [9, 14–18].

5. Preparation procedures and clinical features of PRFs

Despite the high platelet and growth factor ratios provided by PRPs, the observed negativities pushed clinicians to new researches. As a result, PRFs, which are the second generation blood concentrates and have the advantages of being prepared more quickly, have emerged. PRFs appeared mainly as blood concentrates created without using additive anticoagulants. A single, high-speed return is achieved in obtaining the PRF. In the most basic centrifuge method that provides the PRFs, single centrifugation is performed for 12 minutes at 2700-3000 rpm per minute [4, 19–23].

In this way, three layers are obtained:

- Protein Poor Plasma (PPP)
- Platelet Rich Fibrin (PRF)
- Red Blood Cells (RBC)

As studies based on PRF progressed, it was understood that the amount of leukocytes in the content had an important effect on ideal wound healing. This is the reason why leukocyte content is also taken into account in the classification of PRFs. The presence of leukocytes has been found to be effective in obtaining clinical results ideally and achieving lower infection rates. PRFs are structures that contain important cells for healing process of the body, but it is also important that they form a three-dimensional matrix and contain significant growth factors [4, 12, 19–23].

PRFs, of course, did not remain as they first appeared, but were developed in time. PRFs with different contents were obtained by applying different centrifuge methods. The first obtained P-PRF consisted of a solid fibrin matrix structure and did not contain leukocytes. Later, L-PRF appeared, known as leukocyte and platelet-rich fibrin. The A-PRFs were produced by Choukroun, the first revealer of PRFs. This type of PRF has a better handling than other types of PRFs. It is obtained by low speed centrifuge. 14-minute centrifuge process at 1500 rpm is required for the A-PRF acquisition. A-PRFs are blood concentrates containing high amounts of leukocytes. In addition, I-PRFs, an injectable form of PRFs, have emerged. I-PRFs are a type of PRF that can also contain stem cells. To obtain it, centrifuge should be applied for 3-4 minutes at 800 rpm. Another type of PRF is T-PRF. The main difference is that



Figure 1.
PRF clot just after centrifuge.

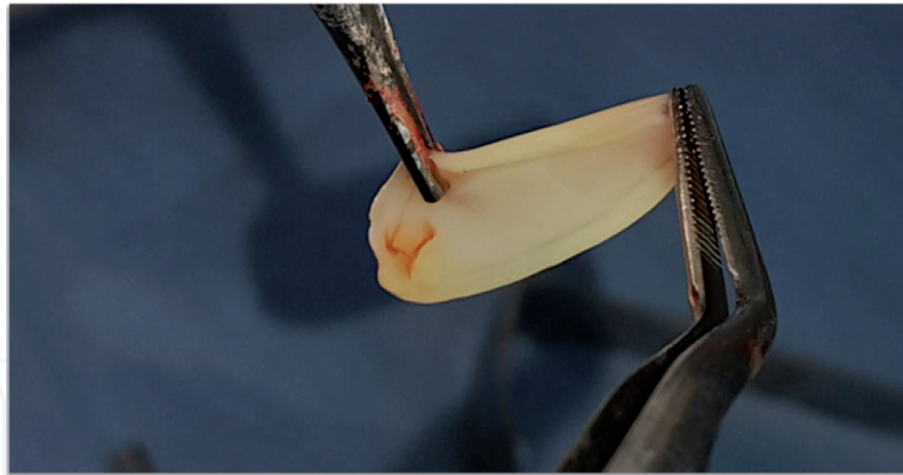


Figure 2.
PRF membrane after pressing PRF clot between two sterile surfaces.

the tubes are made of titanium. According to some studies fibrin matrix obtained in this method is considered to be more successful (**Figures 1 and 2**) [4, 12, 19–23].

PRFs have been of great interest and improved platelet concentrates for many years. The reason for this is of course their advantages. Unlike the PRPs we mentioned earlier, PRFs are produced completely autogenously. There is no need for extra biochemical interventions. It has an easy application procedure. The method is inexpensive since there is no kit requirement or extra process requirement mentioned in PRPs. Since there is no substance added from the outside, fibrinogen turns into fibrin thanks to the thrombin that is found in the blood spontaneously. In this way, the mechanism operates slowly and in accordance with its natural state. The fibrin thus formed is also a close to nature fibrin. Cell migration and proliferation increased compared to PRP. There are studies showing that it positively affects osteoblast activity. Also, when compared with PRP, it is seen that PRFs have higher fibrin content [4, 19–23].

In addition to the many advantageous features described, PRFs also have some disadvantages. Blood is required to be applied. This situation creates anxiety in patients or increases the tendency to refuse treatment. When it is desired to obtain high amounts of PRF, serious blood intake is needed. There is a need for a glass-coated tube to perform the process [19–23].

6. Application fields in dentistry

PRFs have been used in many fields of medicine and dentistry since its occurrence. It is used in general medicine especially in orthopedic field and for the healing of open wounds and ulcers. In dentistry, it is used in endodontics, periodontology and oral and maxillofacial surgery. Main fields of clinical usage are given below [4, 19–23].

ENDODONTICS:

- Can be used for pulp regeneration
- It can be used for apex formation procedures.
- It can be used in the treatment of periapical cysts.

- Can be used as a pulp capping material.

PERIODONTOLOGY:

- It is used to treat intrabony defects.
- It is used to stimulate wound healing after periodontal surgery.
- For coverage of open root surfaces

ORAL and MAXILLOFACIAL SURGERY:

- It is used to protect the socket.
- It is used to prevent alveolitis.
- It is used to ensure the ideal healing of the area after apical surgery.
- It is applied to the peri-implant region to support osseointegration.
- It is used in grafting procedures in bone deficiencies.
- Can be used with or without grafts in sinus lifting procedures.
- It can be used to accelerate recovery after surgery for osteonecrosis.
- It can be used as matrix in biomaterial applications.

7. Biological view of PRFs

Understanding the biological properties of PRFs depends on having information about the content. Also, it is important to know the wound healing process to understand the effects of PRF. The healing of wounds is a process that starts with the hemostasis mechanism and continues with a series of events. The effect of PRFs depends on some key features. The first is the presence of the fibrin matrix. In this way, the fibrin matrix acts as a scaffolding where the cells providing the construction events migrate. The second is the fibrin matrix and the cells in it; secrete chemotactic proteins, cytokines and growth factors. The third is that the fibrin matrix and its factors induce vascular structure formation [4].

The effects of PRFs come from the elements in it. Its structure consists of fibrin matrix and cellular elements. The most important cell in its structure as a cellular element is of course platelets. In addition, its structure includes leukocytes, neutrophils, monocytes and mesenchymal stem cells. Growth factors, which are considered as the most important content of PRFs, are secreted by other cellular elements, especially platelets. The secretion of growth factors and their functions are very important for the functions of PRFs [4].

We know that PRFs provide secretion of many growth factors. These factors and effects are given below;

- TGF- β 1: This molecule is an important inflammation regulator. It is also a very strong fibrosis agent. Its most important tasks are tissue repair, immune modulation and extracellular matrix synthesis. In addition, TGF- β 1 plays an

important role in collagen production. TGF- β 1 also plays a role in restoring epithelialization and connective tissue healing. Transforming growth factor beta is also a critical mediator for bone formation. It stimulates the chemotaxis, cleavage and accumulation of osteoblasts [4, 24–26].

- PDGF: This growth factor known as platelet derived growth factor is important for the proliferation, migration and survival of mesenchymal cells. This growth factor also enables extracellular matrix production to occur during tissue healing. He is also involved in collagen production-demolition mechanisms. PDGF is also a mitogen factor for osteoblast and fibroblast cells [4, 24–26].
- EGF: It stands for epidermal growth factor. The receptors of this factor are expressed in the majority of human cells, including cells that are important in wound healing stages. EGF stimulates the division of mesenchymal stem cells. It also provides chemotaxis in endothelial cells [4, 24–26].
- VEGF: Vascular endothelial growth factor is most commonly secreted by platelets and macrophages. This growth factor is the most active growth factor for vascular regeneration. It provides angiogenesis and the formation of new blood vessels. In this way, blood flow and thus nutrition increase in injured tissues [4, 24–26].
- IGF: Insulin-like growth factor is released when platelets are activated. It induces the differentiation and division of mesenchymal stem cells. IGF is also one of the mediators involved in the regulation of the programmed cell death process. Besides, IGF stimulates proliferation and differentiation in many cells of the body. IGF is also involved in bone matrix formation and replication of osteoblasts [4, 24–26].

8. Clinical applications in maxillofacial surgery

8.1 Sinus lifting

The high healing potential and ingredients of the PRFs have brought to mind the ideas of both increasing the success and shortening the healing time by using them in sinus lifting procedures. Based on these ideas, PRFs were used in sinus lifting procedures, firstly mixed with grafts and then without using grafts. In studies conducted, it has been observed that even though there are no large differences in the amount of bone tissue obtained as a result of mixing graft materials and PRFs, it accelerates the maturation and the wound healing period. In addition, using PRFs mixed with graft materials makes the manipulation of the graft material quite easy. In addition, when PRF membranes are used in sinus lifting procedures instead of other membranes, PRF membranes have been found to be an inexpensive, easy-to-manipulate membrane, so they can be used to close lateral sinus Windows (**Figure 3**). When used for closing the lateral sinus window, it speeds up the healing process and provides good protection for the Schneiderian membrane (**Figure 4**). In cases where the Schneiderian membrane is perforated, PRF membranes are used to make a healing barrier for the perforation zone [27–31].

Although PRFs have the expectation of controlling tissue inflammation with their special biological properties and providing good vascularization in bone tissue, the studies conducted have not yet shown a common understanding of their effects in the literature [27–32].

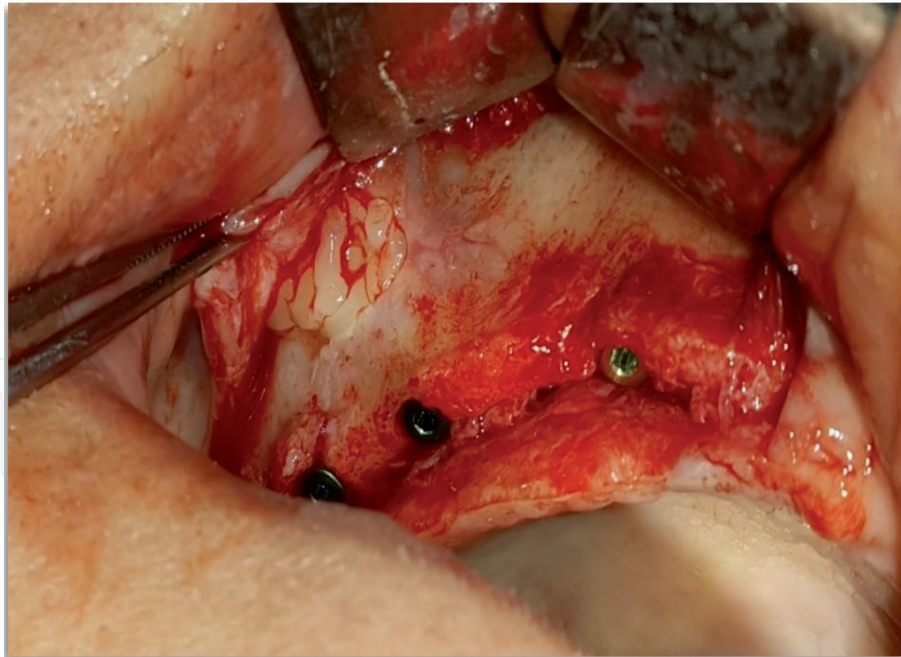


Figure 3.
Application of PRF as a bulk matrix for sinus lifting.



Figure 4.
Application of PRF as a membrane on sinus membrane.

8.2 Alveolar socket preservation

Studies on PRF consider that the use of PRF accelerates epithelialization and vascularization so that wound healing takes place effectively and quickly. However, operations such as tooth extractions and cyst enucleations lead to bone defects after healing process. These defect areas creates esthetic and functional deficiency problems for patients. By using PRFs on healing processes, it has been thought that healing of intra-bony sockets in the alveolar area can be accelerated and bone defect formation can be reduced (**Figure 5**) [32–34].

There are studies that believe that PRFs inserted into the sockets do not make a significant difference in preventing complications in the post-operative period, as some studies show that there is a significant decrease in the frequency of occurrence of alveolar osteitis and bone loss in patients, especially after the 3rd molar surgery. In addition, it is seen that there is no definite opinion about bone gain and remodeling in the literature. However, it is possible to say that the use of PRF improves the patient experience by reducing the frequency of complications such as pain and swelling in the post-operative period. However, its cost is very low, making it attractive to use for post-operative socket protection [34–37].

8.3 Alveolar ridge augmentations

The use of PRF draws attention in augmentation procedures in many fields. PRFs are used for more than one purpose in guided bone regeneration (GBR). PRFs can be used as membranes or preferred to speed up the wound healing process. Studies showed that PRF membranes are successful in closing bone defects. However, the use of PRFs as a barrier membrane in GBR processes alone creates a question. In addition, injecting PRFs into the region in liquid form or placing PRF membranes around the collagen membranes used can stimulate and accelerate soft tissue healing in the region (**Figure 6**). Besides, it can stimulate bone tissue healing. The use of PRF has the potential to increase bone vascularization in areas where GBR is performed. Also, mixing of PRFs with grafts increases the stability and manipulation potential of the grafts in augmentations (**Figures 7–9**) [38–41].

8.4 Implants and PRF

Marginal bone loss may occur following implantation. In order to repair or stop these bone losses, many studies have been performed over the years and different methods have been explained. The clinicians, who thought that marginal bone loss around the implant was completely caused by soft tissue quality and health, thought that PRFs, which are considered to be a very successful material when used in soft tissue augmentation, will completely stop marginal bone loss. There are also studies reporting that the use of PRF in defects around implants, the use of PRFs in flap operations and grafting procedures, yields better results than standard protocols. However, there are no definitive results in the literature regarding the effect of PRF use on hard tissue augmentation and it is stated that more studies are needed [42, 43].

Another area in which PRFs are used in relation to dental implants is their impact on implant stability. In studies conducted on this subject, the stability of the implants was measured by making early resonance frequency analyzes. In the

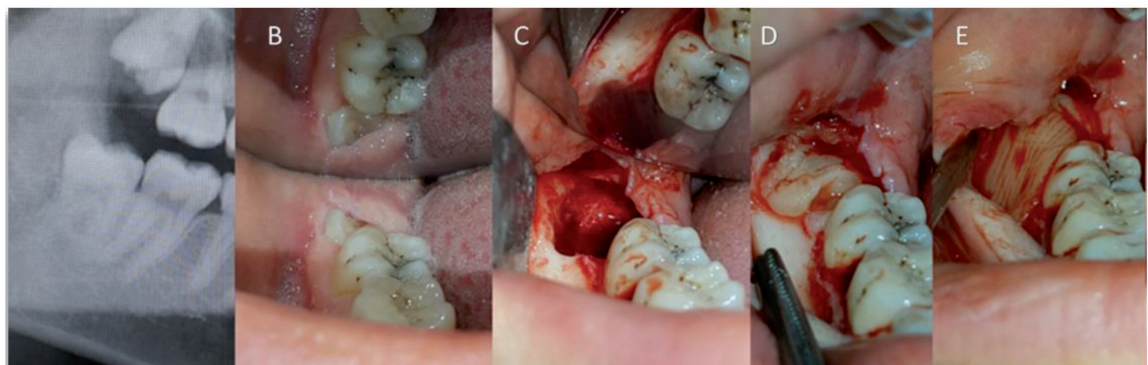


Figure 5.
Using PRF in the extraction socket.

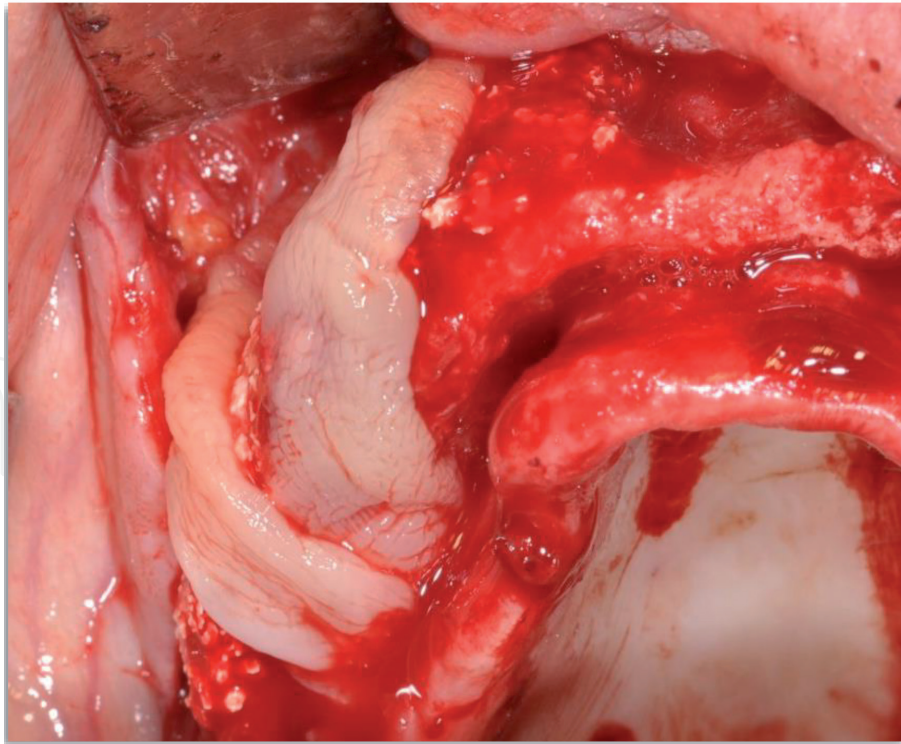


Figure 6.
PRF membranes on the particulated xenografts grafts.



Figure 7.
PRF with bone graft.

studies conducted, it is generally believed that the use of PRF increases the ISQ values of the implants in the early healing period [44–46].

Thanks to the cytokines it contains, it can be used to control inflammation in the inflammatory conditions around the implant as well as in the sockets. Besides, PRFs can be useful in increasing gingival quality and peri-implant periodontal tissue regeneration processes [44–46].

PRFs can be useful in immediate implantation situations where implantation will be performed immediately after extraction. The most important reason for this

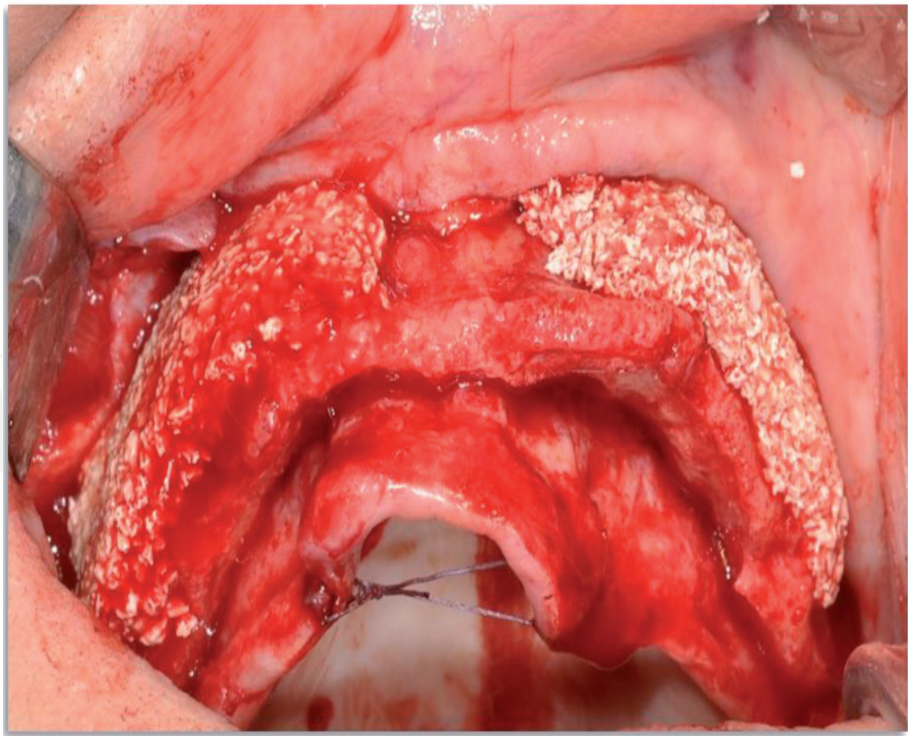


Figure 8.
Alveolar ridge augmentation.

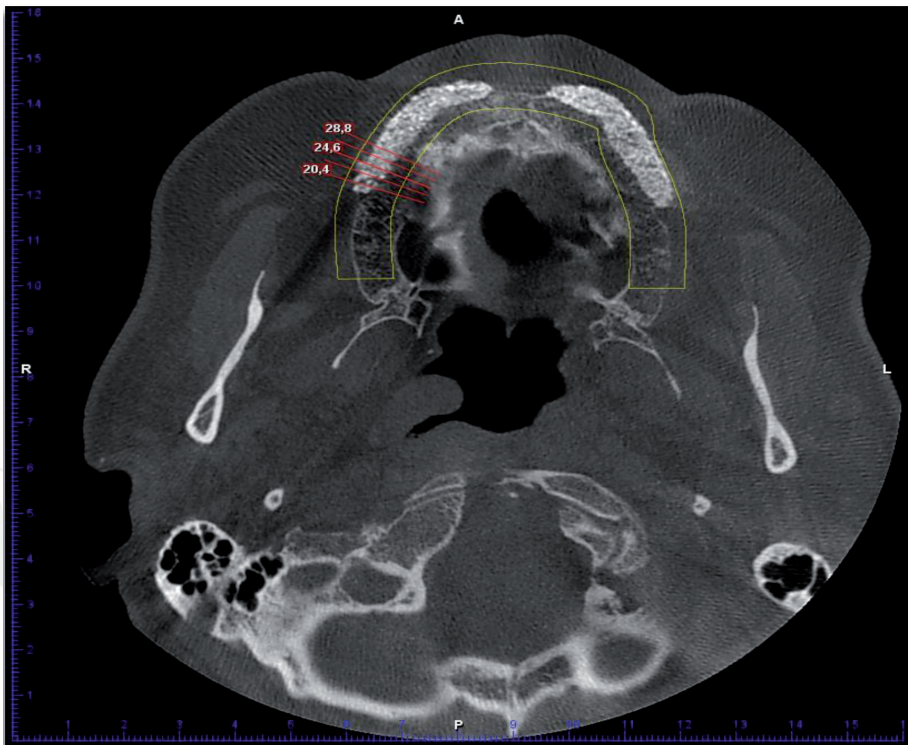


Figure 9.
Grafted area on the CBCCT after 6 months of application.

are the growth factors and cytokines. Thanks to these substances, it will create a high healing potential. In addition, the gaps between the implant and the bone wall can be filled using PRF in immediate implantations. This can only be done by using PRF or by mixing PRF and bone graft materials. However, in cases using collagen membranes, the potential for soft tissue healing can be increased by using PRF (Figures 10–13) [47, 48].



Figure 10.
Deficient bone around implant.

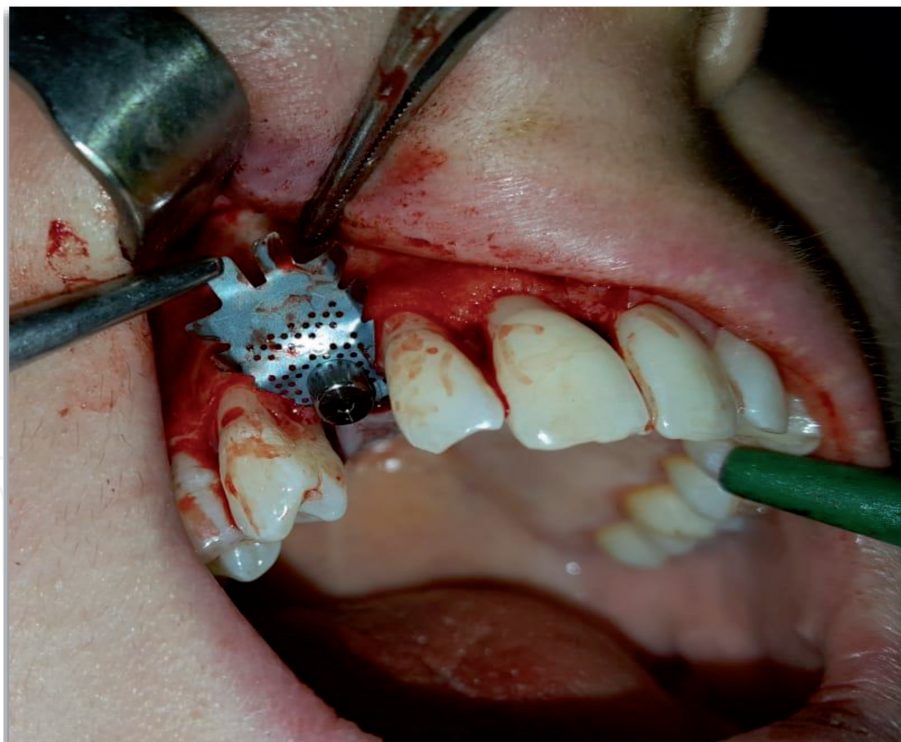


Figure 11.
Titanium mesh application for GBR.

8.5 PRF applications in other fields oral and maxillofacial surgery

In oral surgery practice, oroantral fistulas may occur due to the morphology of the region or due to iatrogenic reasons in the attempts made in the posterior maxilla. Oroantral fistula formation can often occur due to tooth extractions from the region, after cyst excisions or in relation to implant surgery. In the process of



Figure 12.
Application of graft material.



Figure 13.
PRF insertion over Ti-mesh membrane.

closing these fistulas, generally shifted flap techniques are used. However, in recent years, PRF has been used to close these defects. After the region is removed from infected tissues as in other techniques, PRF is applied to the area as thin membranes. In this way, it is not necessary to use outsourced material or use techniques that create serious donor site morbidity [49–51].

Intraoral osteonecrosis scenarios appear due to various medical conditions, especially bisphosphonate group drugs. Generally, some studies in recent years have suggested the use of PRF in osteonecrosis cases, which is tried to be treated

in various ways such as curettage, hyperbaric oxygen therapy, laser stimulations. It has been reported that PRF can act as a barrier membrane and accelerate soft tissue healing around the necrotic areas [52, 53].

PRFs can be used in the surgery of alveolar clefts apart from many other areas mentioned. In addition, it can be used to stimulate healing in patients who have or are likely to experience a healing problem due to medical problems [54].

9. PRF for endodontics

As a result of recent studies, regenerative endodontic therapies have begun to take their place among endodontics procedures. Regenerative procedures are mainly aimed at making the pulp functional by stimulating the pulp tissue that loses its function and cells with the potential for change in it [55, 56].

In the light of the studies, it was seen that PRFs can be used for regeneration in endodontics. For this purpose, in endodontics, PRF can be used for apex closure procedures and for revascularization procedures [55, 56].

In some of the studies conducted, PRF was used with MTA to manage the formation of apex. As a result, it was observed that beneficial results were obtained by using two materials together. The reason for the promising results obtained by using two materials together is that they work synergistically when they are together and thus stem cell and odontoblast stimulation is considered to be more successful [55–59].

Another area regarding the use of PRFs in endodontics is revascularization procedures. It is thought that PRFs can be used as scaffold material for regeneration of necrotic pulp and it is an ideal material as scaffold. The reason for this is, of course, the high amount of growth factors that PRF contains. Thus, it is a very successful skeleton for regeneration. In addition, the coronal pulp removed in regenerative pulpotomy procedures can be replaced with PRF to cover the underlying live pulp. Then it is covered with mineral trioxide aggregate (MTA). PRFs are also included in the apical surgery in the endodontic field. In this field, there are authors who say that the PRFs are successful, as well as the authors who say that the PRFs did not make a significant change. PRFs are thought to be mixed with MTA to create root end barrier. In order to fill the bone cavities formed after apical surgery, good results can be obtained by mixing the bone grafts and PRF [55–59].

10. Using PRFs in periodontology

In the field of periodontology, PRFs are used in the treatment of gingival recessions, intra-bone defects, furcation defects, wound healing of hard and soft tissues, and regeneration of all periodontium tissues. Growth factors used in the periodontium tissues achieve their therapeutic and regenerating properties of tissues by providing differentiation and proliferation inducing effects such as angiogenesis, cementogenesis, differentiation of osteoblasts, anabolic bone formation, etc. Compared with traditional periodontal treatments, it is seen that PRF systems provide very useful results thanks to these properties [60–62].

In addition to periodontal flap applications in teeth with intra-bone defects, successful results are obtained by providing regeneration in hard and soft tissues in the region related to PRF applications. With the use of PRF in intra-bone defects, a decrease in pocket depth scores, an increase in clinical attachment level, and an increase in bone filling of the region are observed, and significant advantages are observed as a result of use alone or in combination with other biomaterials [63, 64].

As with intra-bone defects, PRF applications in furcation defects provide successful results with applications combined with traditional periodontal flap operations. Studies showed that the coronally advanced flap procedure gives more successful results when applied in combination with free connective tissue graft or PRF. Therefore; PRF applications are seen as an alternative to free tissue grafts in periodontal treatments [63, 64].

The use of PRF has been shown to have successful results in patients with aggressive periodontitis. As a result of the combined applications of PRF systems with traditional periodontal surgery, an increase in the level of attachment, decrease in pocket depth and long-term follow-up bone regeneration can be observed in the teeth with aggressive periodontitis [65].

When it comes to gingival recessions, coronally advanced flap procedure with subepithelial connective tissue are among the first periodontal treatments that come to mind. With the widespread use of PRF, the use of PRF instead of subepithelial connective tissue graft is still an area in which studies are ongoing. Compared to the subepithelial connective tissue, it does not create a donor site wound, healing process and preventing patient discomfort during this process are the most important advantages of PRF applications in this area [66, 67].

Another use of PRF membranes in periodontology is that it can be used as a palatal wound bandage or protective membrane after placement of connective tissue grafts. Studies show that it is an effective method to protect the newly formed wound area in palatina and to increase patient comfort by accelerating wound healing in the area [68, 69].

PRF applications are among the most up-to-date and promising areas of periodontology due to their versatile nature. PRFs can be applied alone to the defect surfaces, as well as combined with graft materials or can serve as a thin membrane in regenerative treatments. In addition to these features, they have the feature of being an alternative to connective tissue grafts, thanks to their cellular content, when appropriate. In addition to the aforementioned areas of use, they can function as wound plugs and provide graft stabilization. Considering all these, with its low cost and biological features, PRF applications are effective in many areas in periodontology [63, 64].

11. Conclusion

In many studies, successful results have been obtained from PRFs. It can be said that the most important reason for PRFs success is the growth factors in its own structure. The accelerator and strengthening effect of wound healing is quite obvious with their effect. Besides, it is seen that PRFs have positive effects in regeneration and augmentation procedures. Although more studies are needed on the use of PRFs in some specific areas, PRFs are used quite frequently. In the future, their use seems to increase in all fields of dentistry especially in oral and maxillofacial surgery.

Conflict of interest

The authors declare no conflict of interest.

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