



Techniques of Obtaining BMAC, PRP, and PRF

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The popularity of bioactive surgical additives in oral surgery procedures has seen an increase in recent years. These bioactive additives are used to regulate inflammation and increase the speed of the healing process. These agents augment the wound healing process through anabolic bone formation, angiogenesis, osteoblast differentiation, mitosis, chemotaxis, and other processes that improve the healing environment.

While the entire hard and soft tissue healing process is not completely understood, platelets play a crucial role not only in hemostasis but in wound healing as well. The first mention of platelets' regenerative potential was by Ross et al. in 1974. They described a growth factor from platelets which stimulated the mitogenic response in the bone periosteum during bone repair wound healing. Specific growth factors contained in the

a-granules of platelets (such as platelet-derived growth factor, transforming growth factor- β 1, epithelial growth factor, vascular endothelial growth factor, insulin-like growth factor-I, basic fibroblast growth factor, and hepatocyte growth factor) promote the bone regeneration of oral and maxillofacial bone defects. Platelets also play a role in the host defense mechanism at the wound site by delivering signaling peptides that attract macrophage cells. In addition, platelet concentrates contain leukocytes that synthesize interleukins involved in the non-specific immune reaction. All of which has led to an increase interest in platelet concentrates as bioactive additives. In this chapter we will discuss the three most common regenerative techniques utilizing the platelets as a bioactive additive, the platelet-rich plasma (PRP), the bone marrow aspirate concentrate (BMAC), and the platelet-rich fibrin (PRF).

Bone marrow aspirate and bone marrow aspirate concentrate (BMAC) is a generous source of mesenchymal stem cells and osteoprogenitor cells. Once harvested, the cells can be added to any type of bone graft in order to facilitate osteogenesis. It has been proven that mixing the stem cells from the bone marrow with a biocompatible bone graft will enhance the differentiation and proliferation of stem cells into mature bone. Furthermore, the utilization of allogenic graft acting as a scaffold with the BMAC will avoid the morbidity associated with harvesting autogenous bone and will yield to bone formation.

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The preferred harvest site is either from the anterior or posterior ilium (Fig. 5.1). Bone marrow aspiration from the anterior iliac crest is virtually free from complications. The procedure can be done as an outpatient with local anesthesia, with or without sedation. On the other hand, aspiration from the posterior ilium is usually accomplished under general anesthesia for patients' comfort.

To harvest a sufficient amount of mesenchymal cells, two puncture sites are recommended to aspirate 60–120 ml of bone marrow aspirate. A 2 mm incision is made at the puncture site; blunt dissection is preceded down to the cortex (Fig. 5.2). The sharp trocar punch, with heparin

coating, is pressed through the outer cortex into the marrow space using a twisting motion. Once in the marrow, the trocar is removed leaving the hollow aspiration sleeve inside (Fig. 5.3). Also, a bone core can be harvested using the sharp trocar (Fig. 5.4), and this bone core can be used as an autogenous source of bone graft to the final bone mixture. We use a 20 ml syringe with 0.5 ml of heparin. The 0.5 ml of heparin is injected into the marrow space before the plunger is drawn back to aspirate the bone marrow. The harvested bone marrow must remain anticoagulated. A 4 ml of anticoagulant citrate dextrose-A (ACD-A) is placed in a blood collection bag. Subsequently, each syringe with bone marrow is injected into the bag (Fig. 5.5). Finally, the bone marrow aspirate is drawn from the anticoagulated blood col-

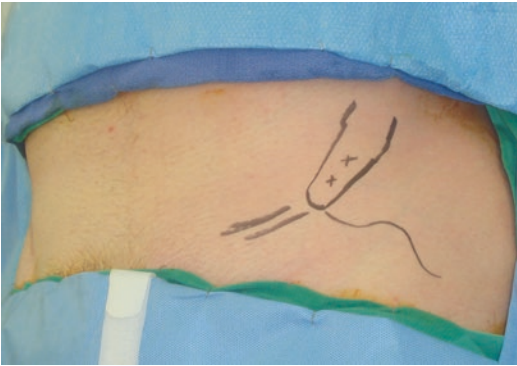


Fig. 5.1 Preparation of anterior ilium for BMAC harvest



Fig. 5.3 Sharp trocar before insertion into the anterior iliac puncture site

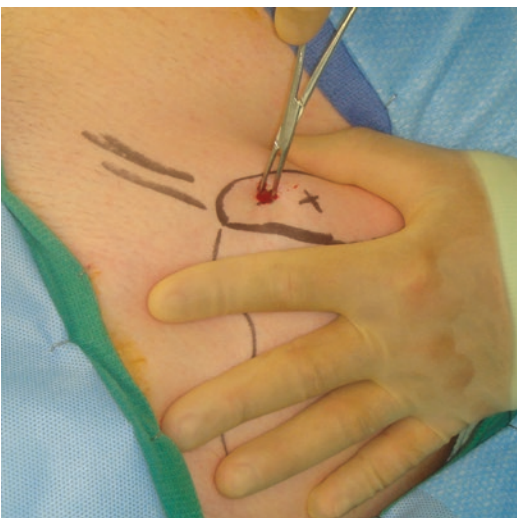


Fig. 5.2 Blunt dissection of the puncture site



Fig. 5.4 Obtaining of the bone core to be mixed with the BMAC

lection bag and placed into the chamber for centrifuging process (Fig. 5.6). The skin incision is then closed with 5-0 fast resorbing gut suture and no dressing is required.

After centrifuging, two fractions are formed into the chamber. First one is the plasma fraction, which contains the adhesion molecules, fibronectin and fibrinogen. The other fraction is the cellular fraction, which contains the mesenchymal stem cells and the osteoprogenitor cells (Fig. 5.7). The cellular fraction can be added to the bone graft to enhance the bone formation (Fig. 5.8). Finally, the plasma fraction will be placed on top of the bone graft to act as a scaffold and improve the osteoconductivity of the graft.

The second technique is the platelet-rich plasma (PRP). This technique is characterized by

obtaining the growth factors from a simple blood drawing obviating the need for any autogenous bone procurement with minimal morbidity. The authors usually combine the PRP with bone grafting materials (autogenous or allogenic) to achieve more predictable outcomes. It was reported in the literature that the PRP will help the bone graft by providing the required tissue adhesion molecules, namely, the fibrin, fibronectin, and vitronectin. In addition, it will amplify the signaling effect of rhBMP-2/ACS.

The technique of harvesting PRP starts with aseptic phlebotomy using a 18-gauge needle



Fig. 5.5 Placement of harvested bone marrow in the blood-collecting bag



Fig. 5.6 Bone marrow aspirate in the chamber after centrifuging

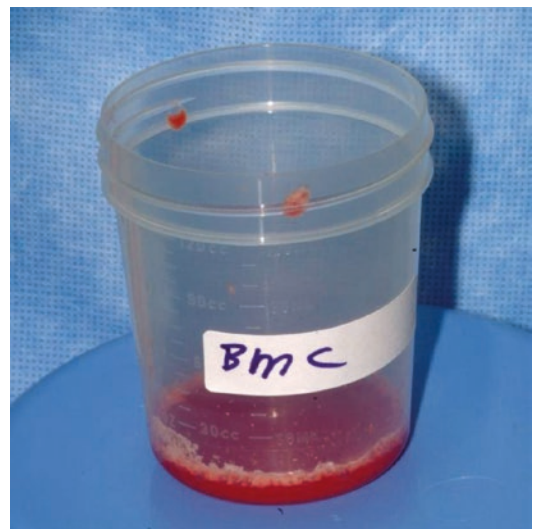


Fig. 5.7 The cellular fraction component of the BMAC



Fig. 5.8 Mixture of the bone graft with the bone marrow aspirate before placement in the recipient bed

to prevent platelets disruption during the aspiration technique. We usually prefer using the antecubital vein because of the ease of use and its large diameter. The syringe should contain Anticoagulant Citrate Dextrose A (ACD-A). According to the manufacturer recommendations, when a 20 ml syringe is used, 2 ml of ACD-A should be placed in the syringe prior to the withdrawal. On the other hand, when 60 ml of autogenous blood is drawn, 7 ml of ACD-A should be used. Once the whole venous blood drawing is completed, we use the SmartPrep device by Harvest Technologies for the centrifuging process. The total time of processing using the SmartPrep device is 14 min.

Once the platelets are separated and concentrated, a distinct layer of the plasma is formed, and this is the platelet-poor plasma (PPP). This layer is drawn separately and can be used as a separate layer to achieve cohesiveness of the grafted material. The other layer contains the anticoagulated PRP that can be stored up to 8 h.

Activation of the PRP is required and this is accomplished by adding calcium to the PRP. The authors' preferred method to add the calcium is by adding 5 ml of 10% calcium chloride to 5000 unit of topical bovine thrombin. Usually, 2–4 drops of this mixture will suffice to activate the clotting cascade of the PRP.

The last technique is the platelet-rich fibrin (PRF), renamed leukocyte PRF (L-PRF) due to its higher leukocyte content, which represents a second generation of platelet concentrates, and its development can be attributed to Choukroun et al. in 2001 [1]. The major advantages of PRF include having completely immune-compatible growth factors collected at relatively no costs without anticoagulants or bovine thrombin or any other gelling agent.

PRF consists of a fibrin matrix of platelets, leukocytes, cytokines, and circulating stem cells. The PRF clot is yielded by a natural polymerization process during centrifugation, and its natural fibrin architecture is responsible for the slow release of growth factors and matrix glycoproteins for 7 or more days. The growth factors released from PRF are transforming growth factor-b (TGF-b), platelet-derived growth factor-AB (PDGF-AB), vascular endothelial growth factor (VEGF), and glycoproteins (such as thrombospondin-1, fibronectin, and vitronectin). Leukocytes in PRF have been shown to be highly important immune cells capable of directing and recruiting various cell types during the wound healing process. Additionally, PRF has a strong induction effect on proliferation of various cells, especially osteoblasts in vitro.

Advanced PRF (A-PRF) is a modification of the L-PRF protocol, whereby the centrifugation G-force is reduced. An increase in total leukocyte numbers within PRF matrix scaffolds was observed in A-PRF. It has also been shown that the release of several growth factors, including PDGF, TGF-b1, VEGF, epidermal growth factor (EGF), and insulin-like growth factor (IGF), was significantly higher in A-PRF compared with L-PRF.

5.1 PRF Preparation

5.1.1 PRF Membrane Fabrication

The PRF process requires the following steps:

1. Collection of whole venous blood in a tube (glass tubes or glass-coated plastic tubes) without anticoagulant. These are red-top tubes.
2. Tube(s) are then centrifuged at
 - (a) 2700 RPM for 12 min for standard L-PRF.
 - (b) 1300 RPM for 8 min for advanced A-PRF.
3. Once centrifuged, three layers are formed (Fig. 5.9): a red blood cell (RBC) base at the bottom, acellular plasma (platelet-poor plasma [PPP]) as a supernatant, and a PRF clot in the middle.



Fig. 5.9 PRF clot in tube post-centrifuge

4. PRF clot is removed from collection tube (Fig. 5.10), and the RBCs are gently scraped off the clot (Fig. 5.11).
5. PRF clots are placed on compression box (Fig. 5.12) to express plasma.
6. Compressed PRF clots (Fig. 5.13) are comprised of a strong fibrin membrane containing leukocytes, platelets, cytokines, and circulating stem cells.

5.1.2 PRF Bone Putty

PRF can be used to enhance particulate bone graft material healing qualities and handling characteristics by creating a putty-like consis-

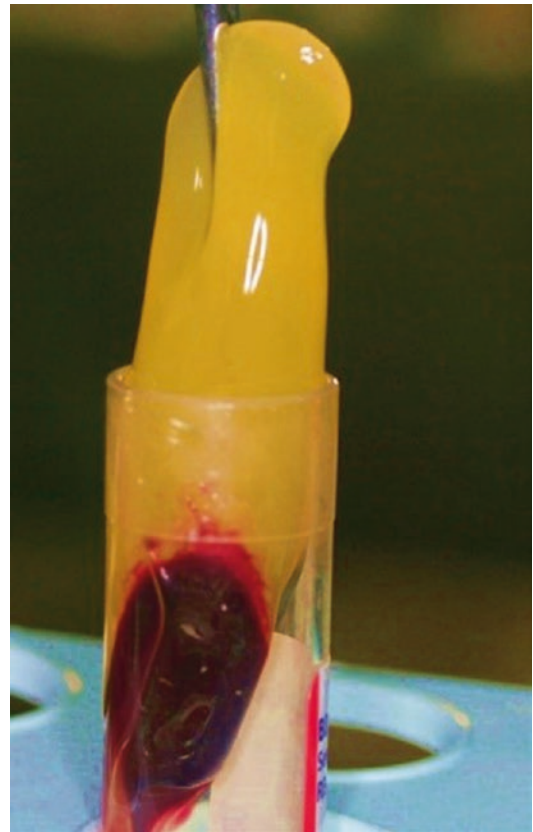


Fig. 5.10 PRF clot removed from tube



Fig. 5.11 Scraping RBC off PRF clot

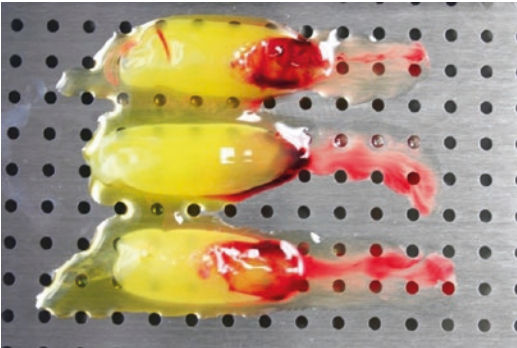


Fig. 5.12 PRF clots on compression box

tenacy. The process to create a “bone putty” is as follows:

1. Collection of whole venous blood in a plastic tube (white top) without anticoagulant for hydration of the particulate bone.



Fig. 5.13 Compressed PRF clots



Fig. 5.14 Supernatant is drawn from the collection tube with a 5 ml syringe

2. Collection of whole venous blood in red-top tubes for PRF membrane fabrication.
3. White-top tube(s) are centrifuged at 2700 RPM for 3 min.
4. Red-top tubes are centrifuged per standard PRF protocol.
5. Supernatant is drawn from the white-top collection tube with a 5 ml syringe (Fig. 5.14).
6. The particulate bone graft is hydrated with the supernatant (Fig. 5.15a, b).
7. Once PRF membranes are formed, the particulate graft is further hydrated with the PRF membrane supernatant (Fig. 5.16a, b).

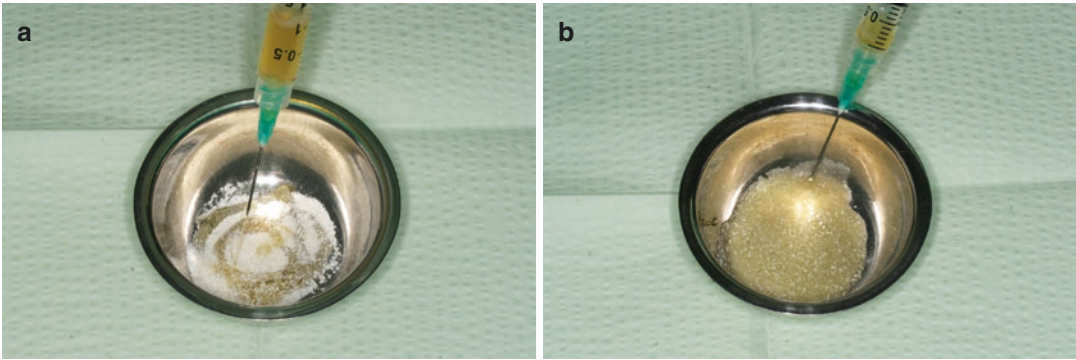


Fig. 5.15 (a, b) Hydrating the particulate bone graft with the supernatant

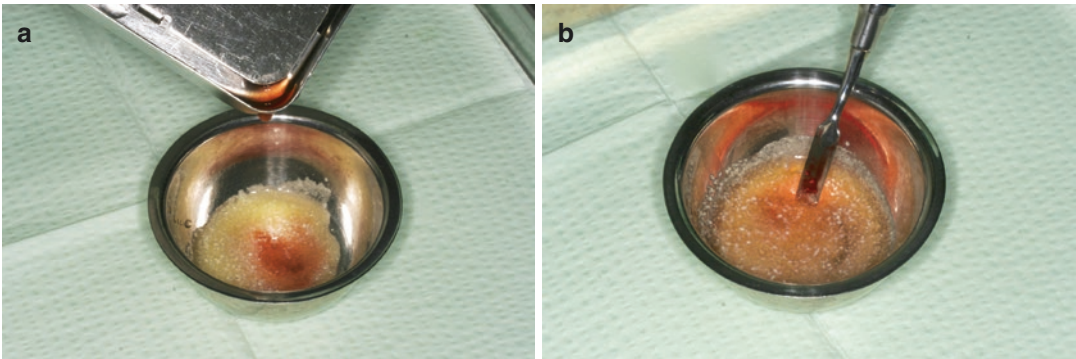


Fig. 5.16 (a, b) Hydrating the particulate bone graft with the PRF supernatant

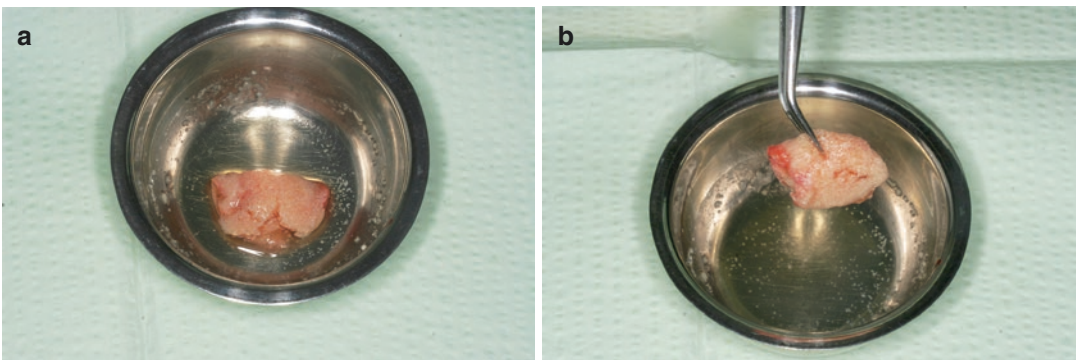


Fig. 5.17 (a, b) Resulting particulate graft putty

This will initiate coagulation and result in a particulate graft putty (Fig. 5.17a, b). Small fragments of PRF membrane can be added to the mix to accelerate the formation of the putty.

5.2 PRF Applications

The compressed PRF clot containing platelets, leukocytes, and growth factors in a strong fibrin membrane will release growth factors for up to 7

days. This biological PRF membrane has multiple oral surgery applications.

5.2.1 PRF in Particulate Grafting Procedures

The formation of a PRF bone putty enhances the handling characteristics of the particulate bone graft and incorporates the enhanced wound healing benefits of PRF. It is of interest to note that PRF placed into extraction sockets has been shown to decrease the rate of complications and infection. This is likely due to the leukocytes present in PRF as leukocytes are the cell type responsible for preventing infiltration by pathogens.

The PRF bone putty can be used in socket graft applications (Fig. 5.18a, b) and in lateral ridge augmentations (Fig. 5.19a, b) where its graft containment characteristics are particularly beneficial. The PRF membrane is also beneficial in these procedures where its physical characteristics are used to create compartmentalized wound healing spaces (Fig. 5.19c).

5.2.2 PRF Membrane as a Palatal Bandage for Free Gingival Graft (FGG) Donor Site

Donor site bleeding, pain, and discomfort are the common problems encountered during FGG procedures. PRF promotes microvascularization and epithelial cell migration to its surface and can be used as a bandage for FGG donor sites (Figs. 5.20 and 5.21). This technique reduces postoperative morbidity and complications associated with donor sites by accelerating the healing process.

Platelet-rich fibrin is a second-generation autologous platelet concentration that is simple and inexpensive to process. PRF contains high amounts of bioactive growth factors to enhance wound healing through increased chemotaxis, proliferation, differentiation, and angiogenesis. It can be used in a variety of oral surgery and implant-related procedures to increase the speed of wound healing and decrease morbidity.

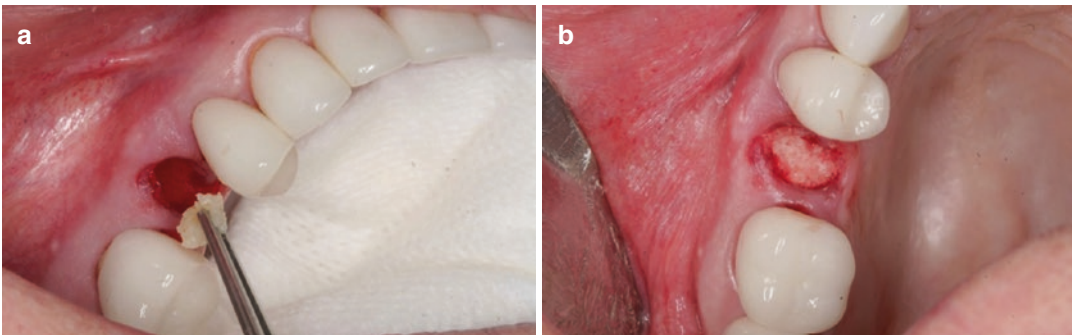


Fig. 5.18 (a, b) Particulate bone graft PRF putty placed in extraction socket

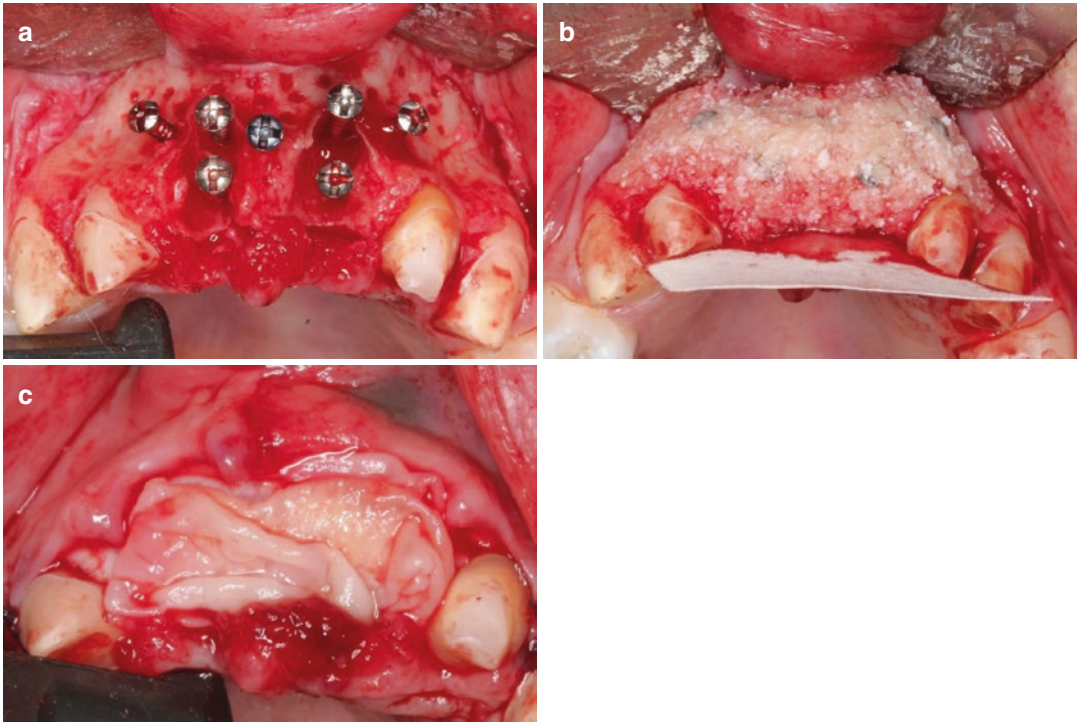


Fig. 5.19 (a, b) Particulate bone graft PRF putty placed in lateral ridge augmentation procedure. (c) PRF membrane placed over bone graft putty to compartmentalize wound healing space

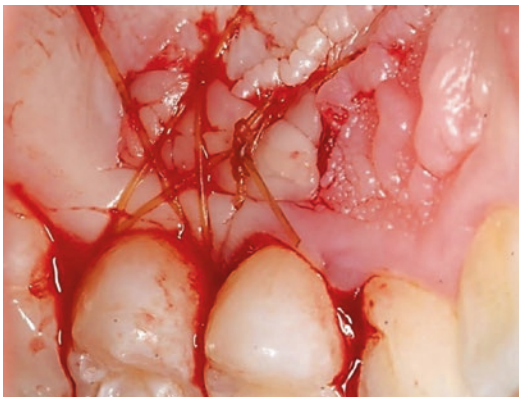


Fig. 5.20 PRF membrane sutured into FGG donor site



Fig. 5.21 1-week healing of FGG donor site

Reference

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