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Abstract: The rich source of autogenous growth factors from platelet rich fibrin (PRF), which is the second generation of platelet concentrate, is considered to promote bone tissue regeneration. Unlike the other platelet concentrates, PRF was defined as an autologous leukocyte and PRF biomaterial, because in this method, platelets and leukocytes are collected with high efficiency such that the growth factors will be able to release gradually during at least 1 week. The aim of this study was to present the clinical results from alveolar ridge preservation using PRF and bone graft. In this study, we reported patient who received PRF as a grafting material together with bone substitute (Bio-Oss) for preserving the alveolar ridge dimension for further prosthetic restoration. The PRF protocol was very simple: A blood sample was taken without anticoagulant in 10 mL tubes which were immediately centrifuged at 3000 rpm for 10 minutes. Prepared “sticky bone” (mixing the bone graft material with A-PRF cut on pieces) was applied in surgical wound and unsighted with PRF membrane. A neovascularization forms through the PRF clot and the epithelial covering developed. Rapid healing of the wound was observed without pain, dryness, or purulent complications. The success of this technique depends entirely on the time gap from blood collection to its transfer for centrifugation, and immediate centrifugation before initiation of the clotting cascade is absolutely essential. The clinical experience confirms that PRF can be considered as a healing biomaterial.

Keywords: alveolar rich preservation, platelet rich fibrin, bone graft

INTRODUCTION

Platelets are involved in the process of wound healing by blood clot formation and with regenerative potential via growth factors released from alpha granules. Platelet concentrates are blood extracts after centrifugation for the concentration of growth factors found in platelets. (Gau et al. 2011) Platelet concentrates can act as bioactive surgical additives that are applied locally to promote wound healing.

Platelet-rich fibrin (PRF) described by Choukroun et al. (Choukroun et al. 2000) is a second-generation platelet concentrate which allows one to obtain fibrin membranes enriched with platelets and growth factors, after starting from an anticoagulant-free blood harvest without any artificial biochemical modification.

The PRF clot forms a strong natural fibrin matrix, which concentrates almost all the platelets and growth factors of the blood harvest (Dohan et al. 2006) and shows a complex architecture as a healing matrix, including mechanical properties no other platelet concentrate offers. It is an autologous biomaterial, and not improved fibrin glue.

Its advantages over platelet-rich plasma (PRP) include ease of preparation, ease of application, minimal expense, and lack of biochemical modification (no bovine thrombin or anticoagulant is required). This considerably reduces the biochemical handling of blood as well as risks associated with the use of bovine-derived thrombin. PRF also contains physiologically available thrombin that results in slow polymerization of fibrinogen into fibrin which results in a physiologic architecture that is favorable to wound healing. (Preeja et al. 2014)

Unlike the other platelet concentrates, PRF was defined as an autologous leukocyte and PRF biomaterial, because in this method, platelets and leukocytes are collected with high efficiency such that the growth factors will be able to release gradually during at least 1 week. (Dohan et al. 2009; Dohan et al. 2006)

It has been shown in different studies that PRF has a proliferative effect on different types of cells such as dental pulp cells, (Huang et al. 2010) human osteoblasts, (Dohan et al. 2009) human gingival and periodontal

ligament fibroblasts, (Chang et al. 2011) dermal prekeratinocytes, and preadipocytes. (Dohan et al. 2009; Wu et al. 2012; Tsai et al. 2009)

This homogeneous fibrin network is considered a healing biomaterial and is used to enhance bone regeneration and soft tissue healing in implant and periodontal plastic surgery procedures, (Dohan et al. 2010) healing of extraction sockets, (Zhao et al. 2011) and treatment of intrabony defects (Chang et al. 2011) and radicular cysts.(Zhao et al. 2011)

Dohan et al. (Dohan et al. 2006; Dohan et al. 2010) stated that PRF has immunological and antibacterial properties, may lead to leukocyte degranulation, and has some cytokines that may induce angiogenesis and pro/anti-inflammatory reactions.

Some clinical applications have been described in oral maxillofacial surgery (Choukroun et al. 2006; Zhao et al. 2011) and in dental implantology. (Diss et al. 2008; Mazor et al. 2009)

In surgical procedures, PRF could serve as a resorbable membrane for guided bone regeneration (GBR) (Chang et al. 2011), preventing the migration of non-desirable cells into bone defect and providing a space that allows the immigration of osteogenic and angiogenic cells and permits the underlying blood clot to mineralize. (Molly et al. 2006.)

CASE REPORT

The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000. Written informed consent was obtained from the patient after a thorough explanation of the nature, risks, and benefits of the clinical investigation and associated procedures.

Under local anesthesia, after incision and flap reflection, on tooth 17 was noticed (Figure 1).



Figure 1. Orthopantomogram X-Ray shows bone loss around the second upper right molar



Figure 2. Preoperative view with expose of bone defect around tooth 17

The PRF protocol is very simple: A blood sample is taken without anticoagulant in 10-mL tubes which are immediately centrifuged at 3000 rpm for 10 minutes. The absence of anticoagulant implies the activation in a few

minutes of most platelets of the blood sample in contact with the tube walls and the release of the coagulation cascades. A fibrin clot is then obtained in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma at the top (Figure 3).

The fibrin clot was easily separated from the lower part of the centrifuged blood. The PRF clot was gently pressed into a membrane between two sterilized plate glass. PRF membranes were minced as graft materials and trimmed as membranes.

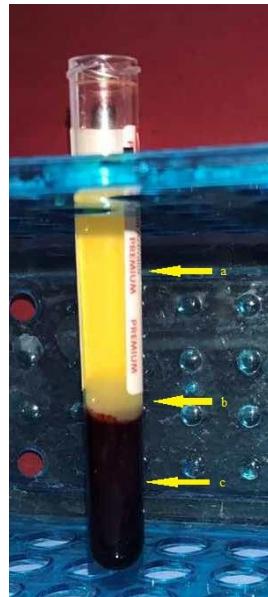


Figure 3. Layers of centrifuged blood samples, tube with three fractions:

- a) acellular plasma at the top,
- b) fibrin clot in the middle of the tube,
- c) just between the red corpuscles at the bottom and.

The cuneiform bone defect was grafted with PRF and bone substitute (Bio-Oss) for preserving the alveolar ridge dimension for further prosthetic restoration. Prepared “sticky bone” (mixing the bone graft material with A-PRF cut on pieces) was applied in surgical wound and unsighted with PRF membrane (Figure 4, 5).

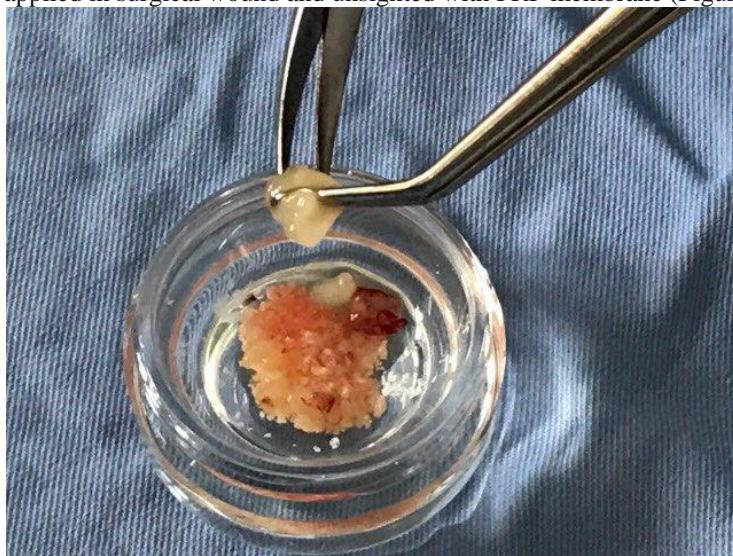


Figure 4. Preparation of sticky bone



Figure 5. Application of “sticky” bone on surgical side for horizontal bone augmentation and guided bone regeneration (GBR)

A neovascularization forms through the PRF clot and the epithelial covering developed. Rapid healing of the wound was observed without pain, dryness, or purulent complications (Figure 6).



Figure 6. Surgical wound after removed sutures

PRF application exhibited the radiographic intensity increase by periapical radiography after 6 months over lesion areas (Figure 7).



Figure 7. X-Ray taken after 6 mounts

After certain period new prosthetic restoration was made.



Figure 8. New prosthetic restoration.

DISSCUSION

PRF was shown to act as a suitable scaffold for breeding human periosteal cells in vitro, which may be suitable for application in bone tissue engineering. PRF also induces the proliferation of various cells in vitro with the strongest induction effect on osteoblasts. (Gassling et al. 2010)

It is hypothesized that PRF has a natural fibrin framework and can protect growth factors from proteolysis. (Lundquist et al. 2008; Dohan et al. 2009) Thus, growth factors can keep their activity for a relatively longer period and stimulate tissue regeneration effectively. PRF can be considered as a natural fibrin-based biomaterial favorable to the development of a microvascularization and able to guide cell migration into wound area.

Some advantages are reported in the literature related to the use of PRF, such as the following:

- Its preparation is a simplified and efficient technique, with centrifugation in a single step, free and openly accessible for all clinicians. (Simonpieri et al. 2012; Dohan et al. 2007)
 - It is obtained by autologous blood sample. (Choukroun et al. 2006)
 - Minimized blood manipulation. (Kang et al. 2011)
 - It does not require the addition of external thrombin because polymerization is a completely natural process, without any risk of suffering from an immunological reaction. (Dohan 2006; Kang et al. 2011)
- It has a natural fibrin framework with growth factors within that may keep their activity for a relatively longer period and stimulate tissue regeneration effectively. (Wu et al. 2012)
- It can be used solely or in combination with bone grafts, depending on the purpose. (Molly et al. 2006; Choukroun et al. 2006)
 - Increases the healing rate of the grafted bone. (Choukroun et al. 2006; Kang et al. 2011)
 - It is an economical and quick option compared with recombinant growth factors when used in conjunction with bone grafts. (Girish et al. 2013)
 - Used as a membrane, it avoids a donor site surgical procedure and results in a reduction in patient discomfort during the early wound-healing period. (Jankovic et al. 2012)

The studies of PRF present it to be more efficient and with less controversies on its final clinical results when compared to PRP. (Simonpieri et al. 2012)

The literature reports some other possible applications of PRF such as:

- In periodontal bone defects: achieving a probing depth reduction and a radiographic defect fills. (Chang et al. 2011)
- In localized osteitis, 90% of osteitis reduction was found in surgical sites of the third molars. (Hoaglin et al. 2013)
- As an adjunct to palatal wound healing after harvesting a free gingival graft. (Kulkarni et al. 2014)
- As a potential scaffold in pulp revascularization procedures of necrotic immature permanent tooth: as it is rich in growth factors, it seems to enhance cellular proliferation and differentiation, augmenting angiogenesis, acting as a matrix for tissue growth, and regulating the inflammatory reaction. (Keswani et al. 2013)
- In multiple extractions to preserve the alveolar ridge height. (Keswani et al. 2013)
- Bone regeneration around immediate implants, inside the alveolar defect. (Simonpieri et al. 2012)

- Reconstruction of large bone defects after cancer surgery. (Reyes et al. 2002)

CONCLUSION

This case report demonstrates that the use of PRF, as the sole grafting material in periodontal osseous defects, is an effective modality in promoting a clinical resolution.

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