INTRODUCTION:

Tissue engineering relates to the cells and biologic mediators in a synthetic or biologic matrix which could be implanted in patients to regenerate tissues. Tissue engineering consists of scaffolds (collagen, bone materials), signaling molecules (growth factors) & cells (fibroblast, osteoblast). These principles of tissue engineering have found widespread application in several branches of dentistry like oral & maxillofacial surgery, periodontics & oral implantology.

Among the great challenges in the development of bioactive surgical additives regulating inflammation and increasing healing, surgeons face complex tissue remodeling and the consequences on healing and tissue survival. The development of platelet concentrate technologies offers a new kind of fibrin adhesive, platelet-rich plasma (PRP) and Platelet rich Fibrin (PRF). The purpose of this article is to describe the second generation platelet concentrate called PRF over traditionally prepared PRP and the simplicity of preparation of PRF compared to PRP.

KEYWORDS: Platelet, Plasma, Platelet Rich Fibrin.

FIBRIN:

Fibrin is the activated form of a plasmatic molecule called fibrinogen. This molecule is present both in plasma and in the platelet α-granules and plays a determining role in platelet aggregation during hemostasis. It is transformed into a kind of biologic glue capable of consolidating the initial platelet cluster, thus constituting a protective wall along vascular breaches during coagulation. Being a soluble protein, fibrinogen is transformed into an insoluble fibrin by thrombin while the polymerized fibrin gel constitutes the matrix of the injured site. Fibrin also provides a matrix for the migration of fibroblasts and endothelial cells that are involved in angiogenesis and responsible for new tissue remodeling.

FIBRIN AND SURGICAL ADDITIVES:

Despite advancements achieved in effective antihemorrhagic surgical techniques, finding hemostatic agents remains a persistent problem. There is a wide variety of hemostatic agents, such as collagen sponges, oxidized cellulose, and cyanoacrylate synthetic adhesives. Over a long period of time, fibrin adhesives have been criticized owing to the fact that they are blood-derived products. More simplified tools inherent to the production of autologous fibrin adhesives have recently been developed with the evolution in similar technologies such as PRP-type platelet concentrates.

PLATELET RICH PLASMA (PRP):

Several studies have shown that bone regenerative
procedures may be enhanced by the addition of specific growth factors. Platelet-rich plasma (PRP) was proposed as a method of introducing concentrated growth factors PDGF, TGF-β, and IGF-1 to the surgical site, enriching the natural blood clot in order to expedite wound healing and stimulate bone regeneration. PRP platelet concentrates are blood-derived products used for the prevention and treatment of hemorrhages due to serious thrombopenia of central origin, such as medullary aplasia, acute leukaemia, etc. They remain of very limited use.

A natural human blood clot contains 95% red blood cells (RBCs), 5% platelets, less than 1% white blood cells (WBCs), and numerous amounts of fibrin strands. A PRP blood clot, contains 4% RBCs, 95% platelets, and 1% WBCs. The classic PRP production protocol requires blood collection with anticoagulant, 2 steps of centrifugation, and artificial polymerization of the platelet concentrate using calcium chloride and bovine thrombin. Since its introduction, PRP has been used in conjunction with different grafting materials in bone augmentation procedures. To date, no conclusions can be drawn regarding the bone regenerative effect of PRP.

TECHNIQUE:

a) Venous blood is taken with anticoagulant.

b) The first centrifugation ("soft spin") allows the blood separation in 3 distinct layers. At the bottom of the tube, the RBC constitutes 55% of total volume. At the top of the tube, the acellular plasma layer made up of circulating plasmatic molecules (fibrinogen) and low in platelets. It is designated platelet-poor plasma (PPP) and constitutes 40% of total volume.

c) Between two layers, an intermediate layer constitutes only 5% of total volume and presents a characteristic buffy aspect that led to it being called "buffy coat." It will compose the major part of the future PRP.

d) Using a sterile syringe, aspiration of PPP, PRP, and some red blood corpuscles to be done. Then the material is transferred to another tube, without anticoagulant. This second tube will then undergo another centrifugation faster than before.

e) With a syringe, the major part of the PPP to be discarded, leaving just enough serum to place the concentrated platelets in suspension. The unit is then gently shaken to obtain a ready-to-use PRP.

f) PRP is then mixed with bovine thrombin and calcium chloride at the time of application, with the help of a mixing syringe. This polymerization will constitute a fibrin matrix with particularly interesting hemostatic and adhesive properties.

PLATELET RICH FIBRIN (PRF):
Platelet-rich fibrin (PRF), developed in France by Choukroun et al. (2001), is a second generation platelet concentrate widely used to accelerate soft and hard tissue healing. Its advantages over the better known platelet-rich plasma (PRP) include ease of preparation and application, minimal expense, and lack of biochemical modification (no bovine thrombin or anticoagulant is required).

PRF is a strictly autologous fibrin matrix containing a large quantity of platelet and leukocyte cytokines. With its strong fibrin architecture and slow release of growth factors and glycoprotein over several days this natural bioactive membrane can enhance soft and hard tissue healing while protecting both surgical and grafted sites.

PREPARATION AND CLINICAL APPLICATIONS OF PRF:
PRF preparation requires an adequate table centrifuge and centrifugation done @ 2700 rpm for 10 minutes (Fig. 1), and a 24 gauge butterfly needle and 9 ml blood collection tubes. Blood is drawn into the tubes without anticoagulant and is immediately centrifuged. Within a few minutes, the absence of anticoagulant allows activation of the majority of platelets contained in the sample to trigger a coagulation cascade. Fibrinogen is at first concentrated in the upper part of the tube, until the effect of the circulating thrombin transforms it into a fibrin network. The result is a fibrin clot containing the platelets located in the middle of the tube, between the red blood cell layer at the bottom and acellular plasma at the top (Fig. 2). Pliers are inserted into the tube to gently grab the fibrin clot with attached RBC's (Fig. 3). Fibrin clots are transferred to sterile metal surface and RBCs are gently scraped away and discarded. The PRF clot is then placed on the grid in the PRF Box (Fig. 4) and covered with the compressor and lid. The PRF Box was devised to produce membranes of constant thickness that remain hydrated for several hours and to recover the serum exudate expressed from the fibrin clots which is rich in the proteins vitronectin and fibronectin. Figure 5 and 6 shows formation of PRF plug for better handling. Figure 7 shows complete coverage of graft and crest with 4 to 6 PRF membranes. Figure 8 shows placement of maxillary implants in reconstructed ridge. Figure 9 shows formation of healthy peri implant soft tissue.

DISCUSSION:
PRF is a matrix of autologous fibrin, which are embedded a large quantity of platelet and leukocyte cytokines during centrifugation. The intrinsic incorporation of cytokines within the fibrin mesh allows for their progressive release over time (7-11 days), as the network of fibrin disintegrates. PRF in the form of a platelet gel can be used in conjunction with bone grafts which offers several advantage including graft stabilization, bone growth and maturation, wound healing and hemostasis, and improves the handling properties of graft materials. The easily applied PRF membrane acts much like a fibrin bandage, serving as a matrix to accelerate the healing of wound edges.
According to Simonpieri et al., the use of this platelet and immune concentrate during bone grafting offers the following four advantages: First, the fibrin clot plays an important mechanical role, with the PRF membrane maintaining and protecting the grafted biomaterials and PRF fragments serving as biological connectors between bone particles. Second, the integration of this fibrin network into the regenerative site facilitates cellular migration, particularly for endothelial cells necessary for vascularization and survival of the graft. Third, the platelet cytokines (PDGF, TGF-β, IGF-1) are gradually released as the fibrin matrix is resorbed, thus creating a perpetual process of healing. Lastly, the presence of leukocytes and cytokines in the fibrin network can play a significant role in the self-regulation of inflammatory and infectious phenomena within the grafted material. From a fundamental point of view, it is still difficult to know if the addition of a fibrin clot really permits enhancement of new bone deposit. PRF contains platelet growth factors as well, but these cytokines seem to have a secondary rule in the bioactivity of PRF. Therefore, PRF does not appear to enhance cellular proliferation in the long term, but may play an important role in the revascularization of the graft by supporting angiogenesis. It also provides a significant postoperative protection of the surgical site and seems to accelerate the integration and remodeling of the grafted biomaterial.

**Use of PRF:**
RF is used in soft tissue repair, for better healing and osteointegration in implant surgeries, plastic surgery, cartilage reconstruction and enhances bone formation.

**CONCLUSION:**
Early publications and clinical experience seem to indicate that PRF improves early wound closure, maturation of bone grafts, and the final esthetic result of the peri-implant and periodontal soft tissues. Literature pertaining to PRF was found in French and the material is being widely used in France. This biomaterial is easy and inexpensive to produce and therefore its systematic use during oral and maxillofacial surgery must be considered as relevant clinical option. The popularity of this material should increase considering its many advantages. In future, more histologic evaluations from other parts of the world are required to understand the benefits.

**REFERENCES:**
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Corresponding Address:
Dr. Shouvik Chowdhury
Email: lifeline143@gmail.com
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