

## EFFECT OF PLATELET RICH FIBRIN AND INJECTABLE PLATELET RICH FIBRIN ON BONE HEALING (COMPARATIVE STUDY ON SHEEP)

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### ABSTRACT

**Objective Aim of study:** This study aims to compare the effect of the PRF , i-PRF on bone defect healing. defect

The Platelet-rich fibrin (PRF) was first developed in France by Choukroun in 2001, an autologous biomaterial was developed that consists of leucocytes and platelet-rich fibrin. (PRF) (Choukroun et al 2001A more recent iteration of platelet-rich fibrin, known as injectable platelet-rich fibrin, has been developed to be utilized in a flowable form. This variant can be prepared at varying speeds and durations. Both platelet-rich fibrin forms have the potential to aid in the regeneration of both soft and hard tissues in areas affected by damage. The presence of bone abnormalities can result in both aesthetic and functional impairments, hence rendering dental rehabilitation more challenging. The aim of this study is to histologically evaluate the process of hard tissue healing in bone defects that have been treated with platelet-rich fibrin (PRF) and its injectable version, known as i-PRF.

**Results :**The presence of inflammation was noted during the initial weeks following surgery, accompanied by a limited proliferation of osteoblasts in platelet-rich fibrin (PRF) compared to injectable platelet-rich fibrin (i-PRF). In the 16th week, the presence of osteoblasts and fibrosis was seen, indicating the development of new and mature bone.

**KEYWORDS:** PRF, i-PRF, bonehealing, hardtissue

### 1.INTRODUCTION

**B**lood is composed of plasma, a combination of many types of cells, and platelets, which are biologically active fragments of cells. Thrombocytes play a crucial part in the process of coagulation, serving to mitigate excessive blood loss in the context of venous injuries. These structures possess a multitude of cytokines and growth factors that exert an influence on the process of bone regeneration and the maturation of soft tissue. Platelet-derived growth factor AB (PDGF-AB), transforming growth factor-1 (TGF-1), and vascular endothelial growth factor (VEGF) are the principal growth factors that are found within platelets. (Anitua E, et al 2006).

The Platelet-rich fibrin (PRF) was first developed in France by Choukroun in 2001 as an autologous biomaterial which contains leucocytes and platelet-rich fibrin (PRF) (Choukroun et al 2001). In contrast to alternative platelet-rich

products, this approach does not necessitate the use of anticoagulant agents or bovine thrombin. (or any other gelling agent) (Dohan DM, et al 2006). Successful clinical results have been stated with PRF (Sharma A and Pradeep AR 2011), The injectable platelet-rich fibrin (I-PRF) is a type of biomaterial created from the patient's own blood. It is considered a second generation biomaterial and shares similarities with platelet-rich plasma (PRP) in terms of its fluid consistency. However, I-PRF also possesses a three-dimensional fibrin meshwork structure, similar to that of a PRF clot. In addition to platelets and their associated growth factors, injectable platelet-rich fibrin (PRF) mostly contains collagen type-1 and lymphocytes, along with their respective growth factors. ( Choukroun J.et.al.)

### 2.MATERIALS AND METHODS

#### 2.1 Protocol for PRF preparation

The blood sample is carefully transferred into a

10ml glass test tube, devoid of any anticoagulant, and promptly subjected to centrifugation at a speed of 3000 revolutions per minute (rpm) for a duration of 10 minutes. The centrifugation process involves a series of biological stages in which thrombin, present in the circulating blood, catalyzes the conversion of fibrinogen into fibrin. Subsequently, the resulting fibrin is positioned at the central region of the test tube. The findings encompass three distinct layers: an uppermost layer consisting of platelet poor plasma, a middle layer composed of PRF clot, and a bottom layer comprising red blood cells. PRF can be acquired in the form of a fibrin clot. The PRF clot can be removed from the test tube by employing a sterile tool resembling a pair of tweezers. Following the lifting procedure, the layer of red blood cells (RBCs) that is attached to the platelet-rich fibrin (PRF) clot can be carefully detached using a sterile pair of scissors. (Mufti and Sonam 2017)

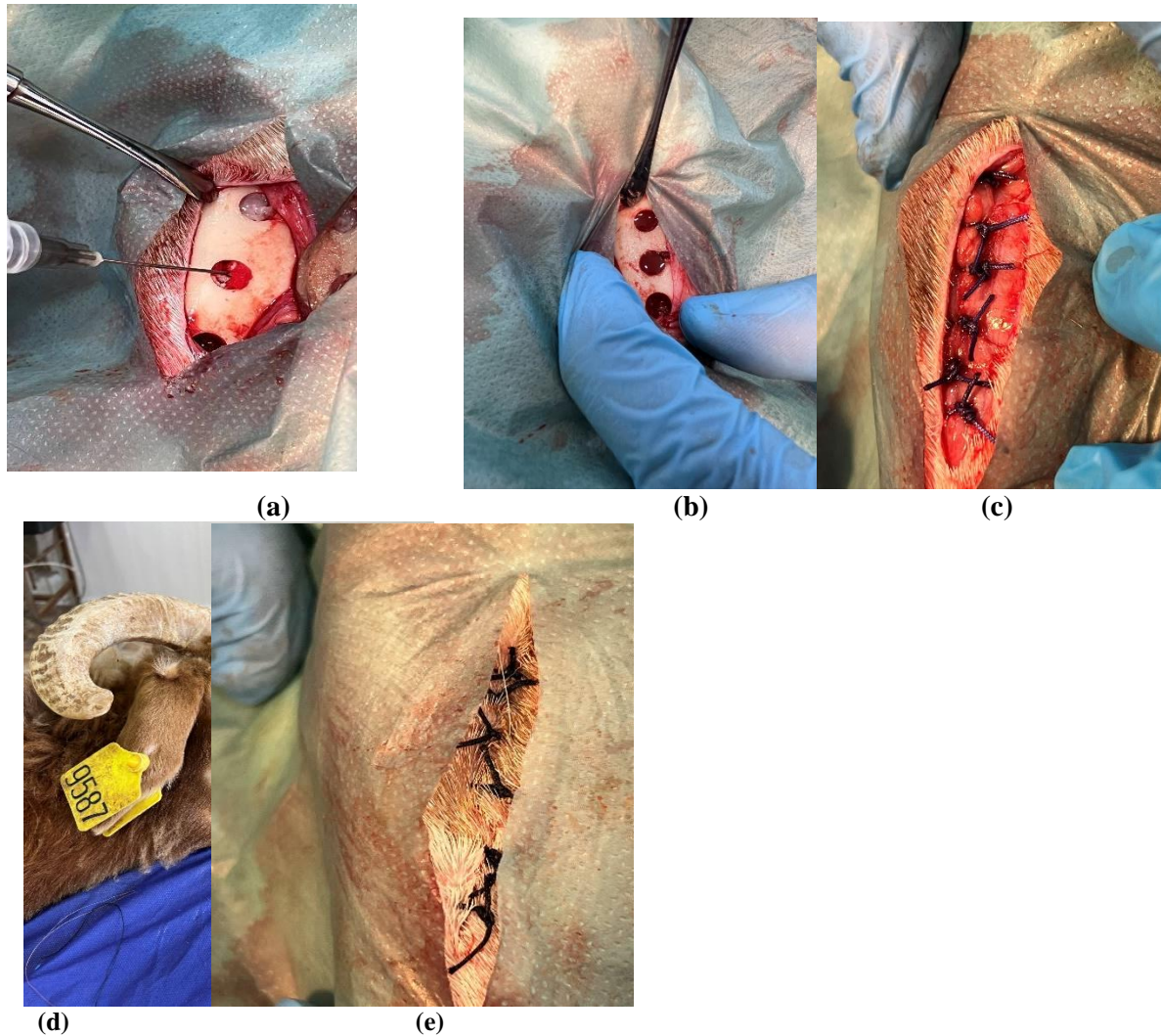
### **2.2 PREPARATION PROTOCOL of i -PRF**

Autologous blood will be collected into polypropylene tubes without the inclusion of any exogenous anticoagulants. The sample was thereafter subjected to centrifugation for a duration of 3 minutes at a rotational speed of 700 revolutions per minute (rpm). Plastic tubes exhibit a hydrophobic surface characteristic that hinders the efficient activation of the coagulation process. Consequently, within the initial 2-4 minutes of centrifugation, the upper zone of the tube is reached by the clotting factors and platelets of the

blood, which are necessary for the production of the platelet concentrate. The plasma and platelets, which are distinct entities, are co-located in the upper layer and exhibit a pale yellow hue. They are commonly administered in injectable format. (Miron R. J. et al, 2017)

### **2.3 Surgical Procedure:**

Blood samples from 10 healthy sheep their age ranged between (6 to 8) months with body weight ranged between (25 to 35) kg. The sheeps are housed indoors in concrete stalls in the animals' farm of the College of Veterinary Medicine/ University of Dohuk two weeks before surgery to become accommodated to the farm condition. The sheep are subjected to a thorough clinical examination and complete blood examination to ascertain their health status. They must to be healthy and free of diseases. The blood samples have been used to confirm the protocol for formation of PRF and i-PRF to develop the experiment. The mucoperiosteal flaps were made and making three incisions . An incision was made (about 3 cm in length and about 1 cm below and parallel to the mandible). After that a dissection to expose the lateral side of the mandible using the blunt dissector, reflecting the periosteum by a mucoperiosteal flap. Then 3 small cavities of 2 cm in depth and 3 cm in diameter will drill into right side of the sheep mandible with straight handpiece and standard bur under copious irrigation with normal saline (Figure 1), a space of about (1cm) was left between the holes.



**Fig.( 1 ) :-** shows a-e steps of the surgical operation from making the three small holes to the final suture

### 3.RESULTS

**3.1Clinical Results :** In total, 15 defects in 5 sheeps were collected and histologically examined. All the animals were healthy before being euthanized. No dropouts were experienced, and no complications or adverse events were observed at any stage of the research. (Figure 2 )

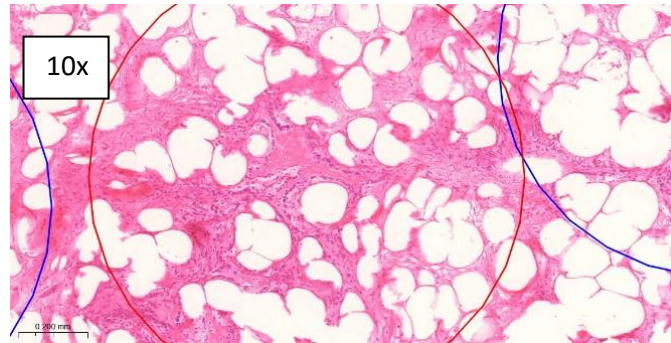
**3.2Histological Findings:** After the slides have been ready for histopathological examination.

Hematoxylin eosin staining was applied to the slides to display cytoplasmic, nuclear, and extracellular matrix features.

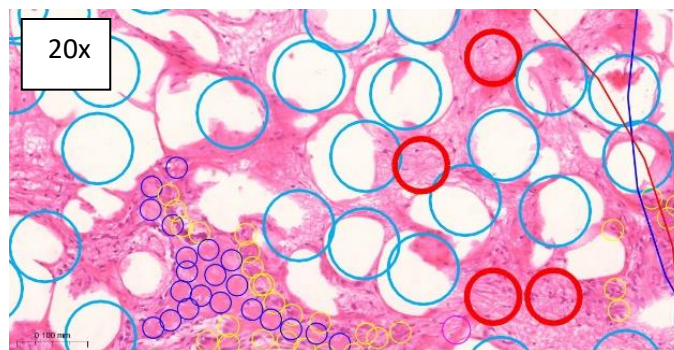
In this study an inflammatory response and osteocytes proliferation was observed after 1 week and 16 weeks

The different type of cells were encircled in different colored circles to differentiate the most proliferated cells .

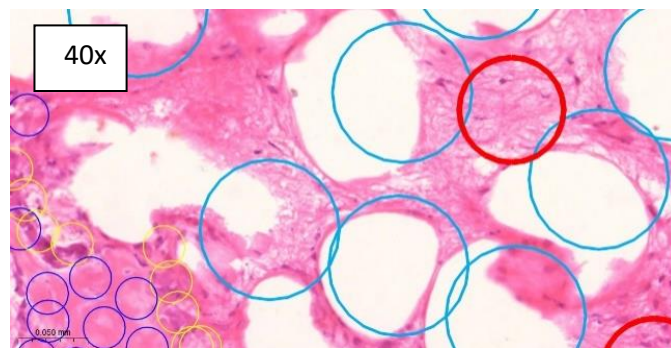
● Fibrosis ● inflammation ● mature bone ● osteoblasts ● osteoclast ● new bone



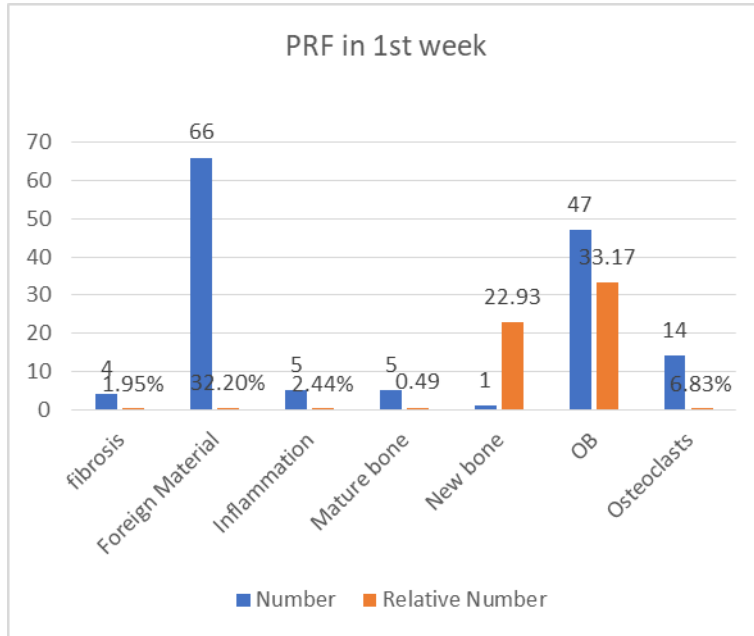
**Fig.(2) : -(a)** 10x using PRF after 1 week



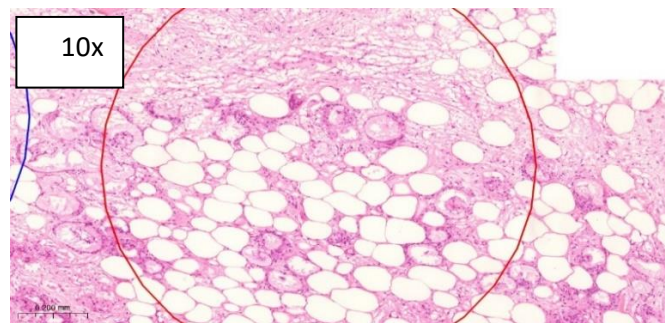
**Fig.(2) : -(b)** 20x Presence of the PRF material with inflammatory cells and few new bone and osteoblasts



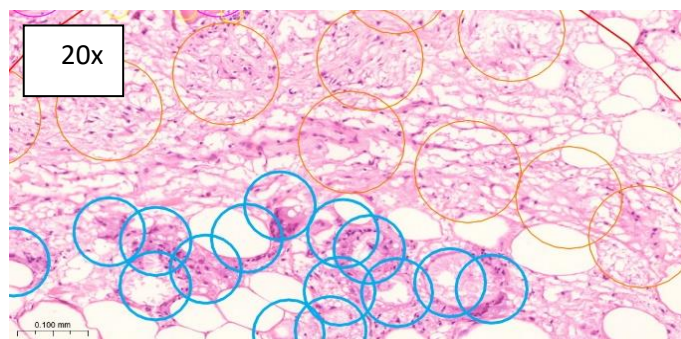
**Fig.(2) : -(c)** 40x shows the PRF and inflammation



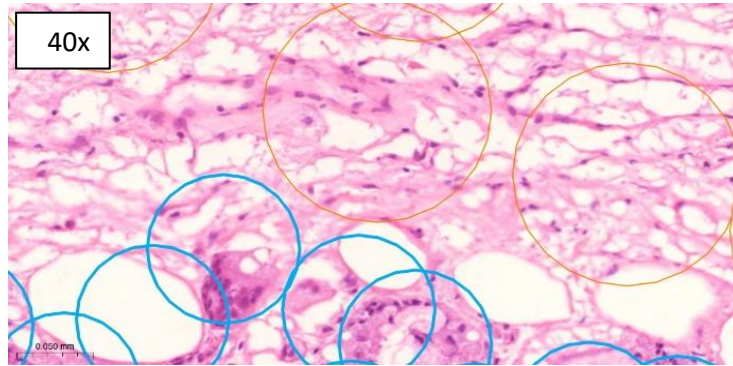
The histological section of the operation site with PRF in the first week shows foreign material and few inflammatory cells with little shows osteoblasts and new bone.



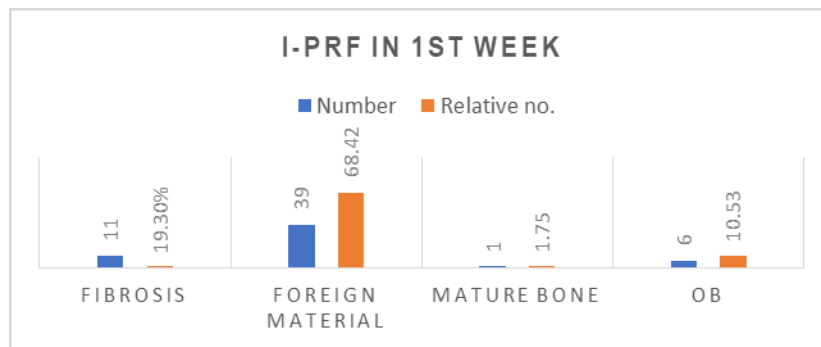
**Fig.(3) : -(a) 10x using i-PRF after 1 week**



**Fig.(2) : -(b) Fig.(2) : -(a) Fig.(2) : -(a) 20x shows presence of i-PRF material with fibrous tissue.**

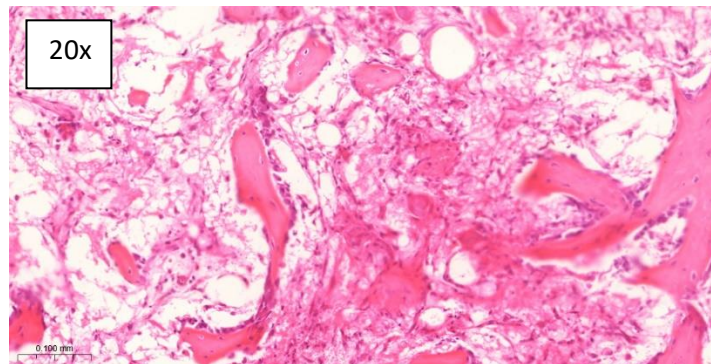


**Fig.(2) : -(c)** 40x shows the foreign material with fibrous tissue



In the same first week but with using i-PRF the histological section shows the foreign material more obvious the the PRF gel form with least amount of osteoblasts in mature bone in comparison with the PRF.

● Fibrosis ● inflammation ● mature bone ● osteoblasts ● osteoclast ● new bone



**Fig.(4) : -(a)** 20x controlled healing in the 1<sup>st</sup> week

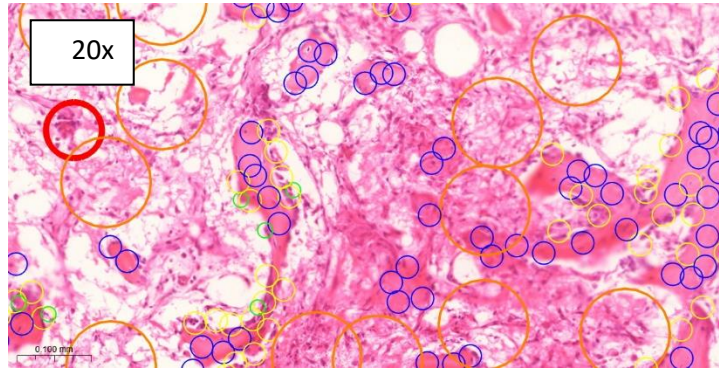


Fig.(4) : -(b) 20x Controlled healing shows inflammation with fibrosis and mature bone

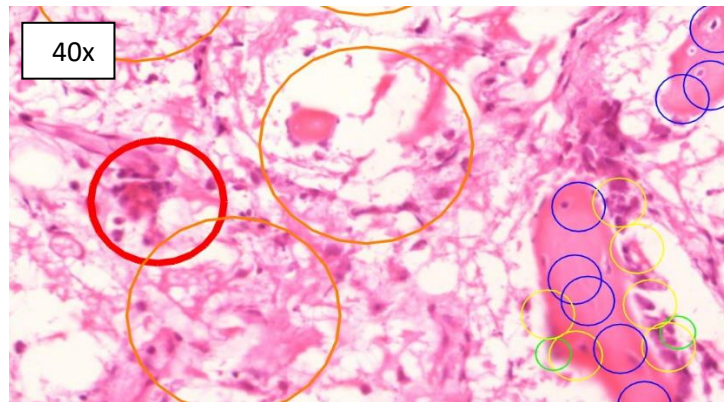
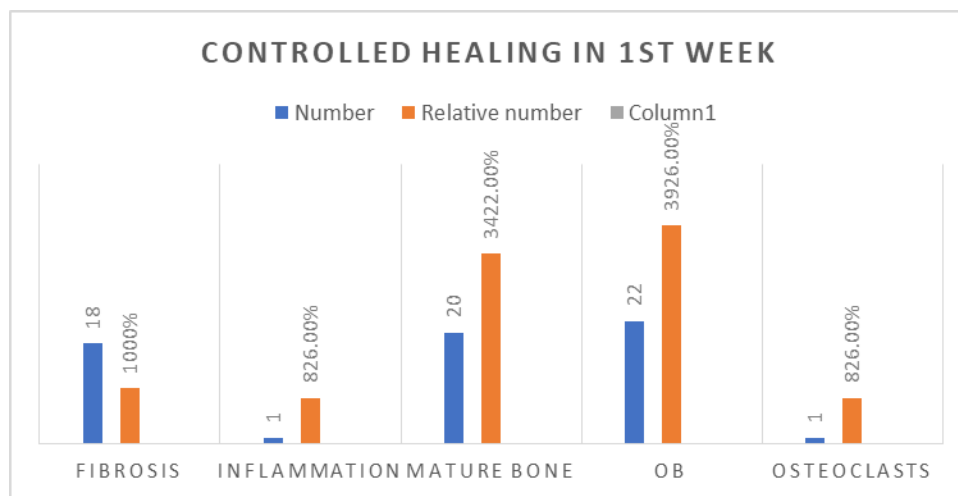
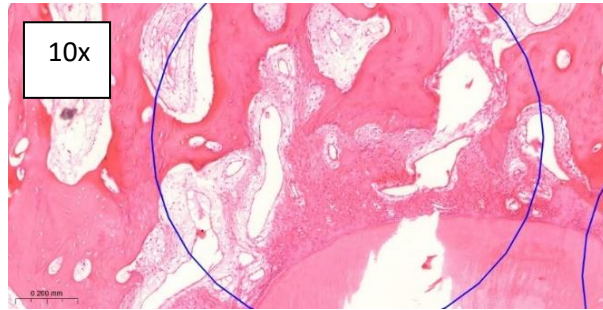


Fig.(4) : -(c) 40x shows the inflammation cells more clear

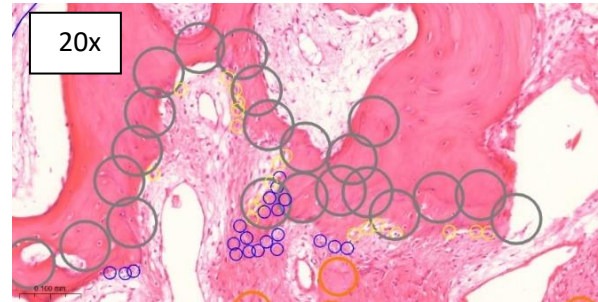
category	Number	Relative number
fibrosis	18	10%
inflammation	1	8.26%
Mature bone	20	34.22%
OB	22	39.26%
osteoclasts	1	8.26%



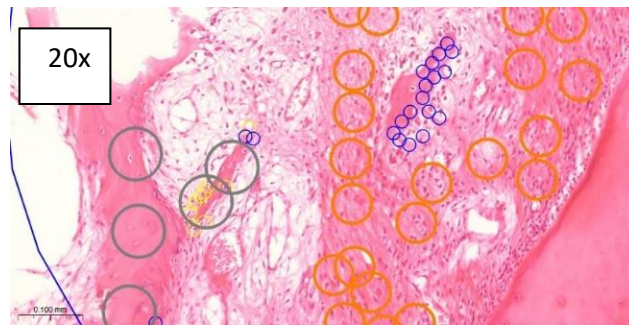
Controlled healing in the first week shows more fibrous tissue than that slides with foreign material



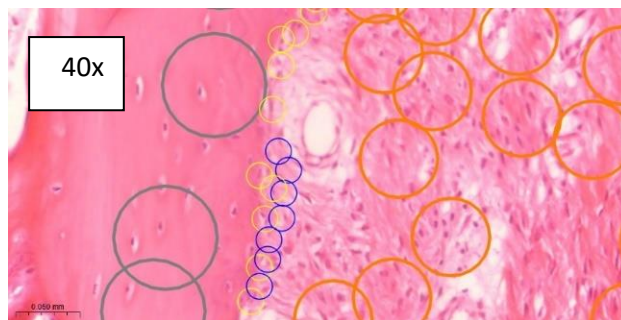
**Fig.(5) : -(a)** 10x PRF used in this sample after 16 weeks



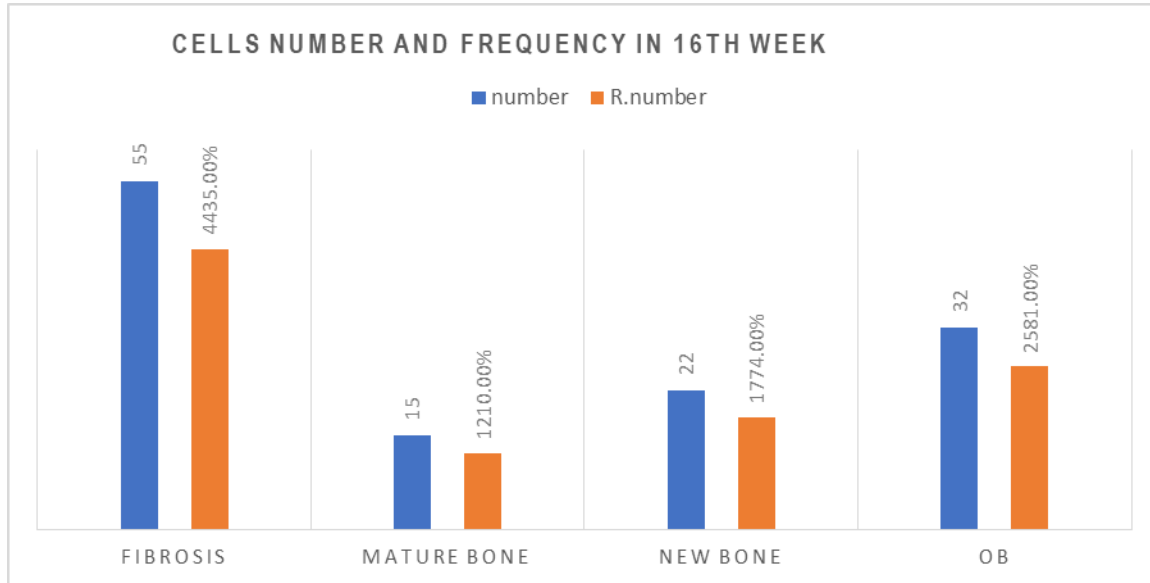
**Fig.(2) : -(b)** 20x shows formation of new bone



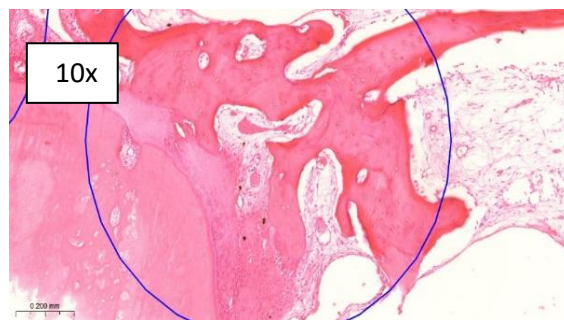
**Fig.(2) : -(c)** 20x also presence of fibrosis with osteoblasts



**Fig.(2) : -(d)** 40x this slide shows continuous formation of ne bone with presence of osteoblasts



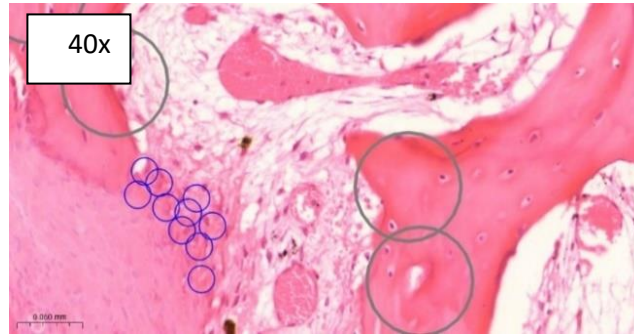
In the 16<sup>th</sup> week the section used in the operation site of PRF shows large amount of fibrous tissue and formation of new bone with more osteoblasts proliferation.



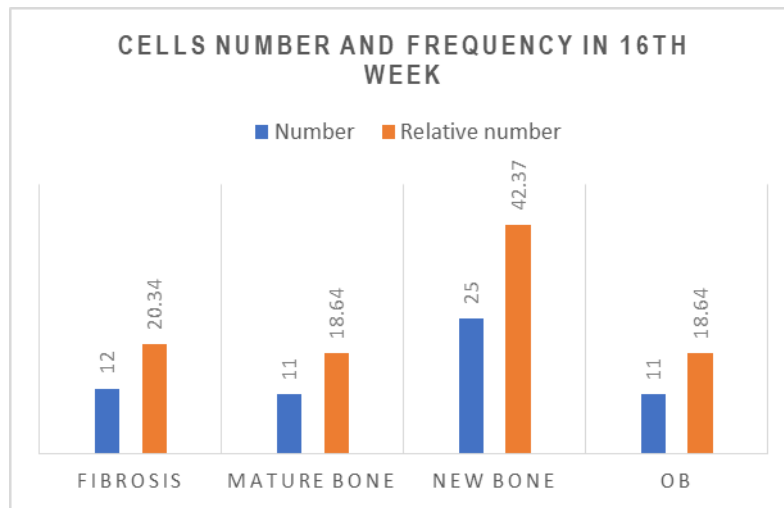
**Fig.(6) : -(a)** 10x i-PRF used in this sample after 16 weeks.



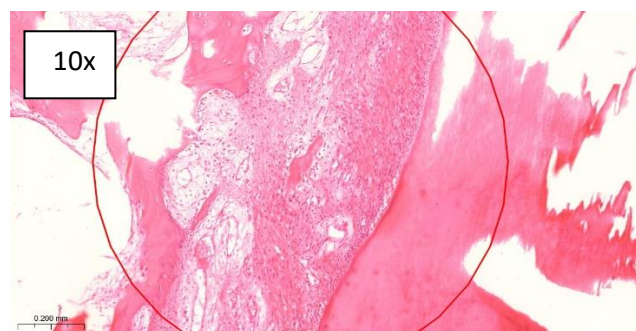
**Fig.(6) : -(b)** 10x shows formation of new bone with presence of mature bone.



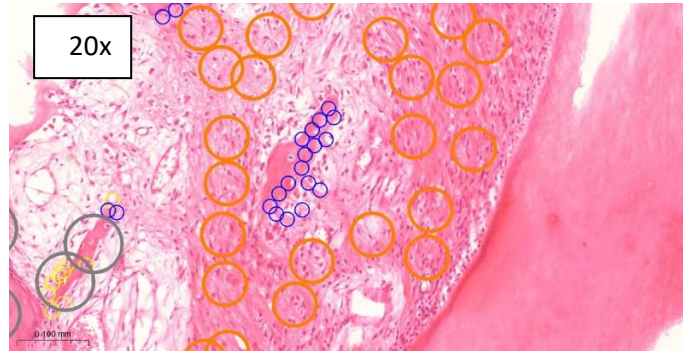
**Fig.(6) :** -(c) 40x shows new bone formation



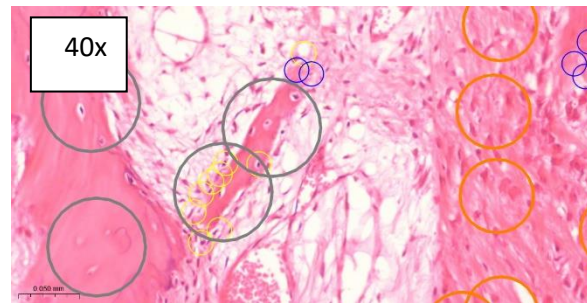
The new bone formed with using of i-PRF in the 16<sup>th</sup> week is more obvious than that with the PRF gel form with least amount of fibrosis and osteoblasts.



**Fig.(7) :** -(a) 10x controlled healing after 16 weeks with fibrous tissue formation and new bone with osteoclastic activity

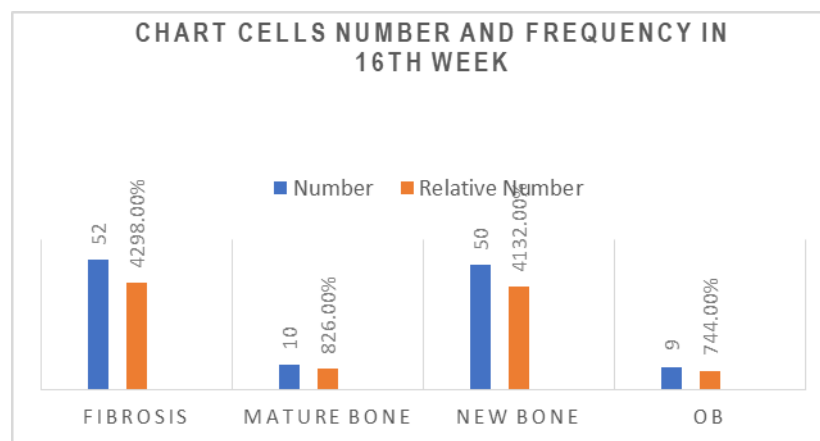


**Fig.(7) : -(b) 20x shows mature bone with slight osteoblastic activity with formation of fibrosis**



**Fig.(7) : -(c) 40x focused on the mature bone and osteoblasts**

category	Number	Relative number
fibrosis	52	42.98%
Mature bone	10	8.26%
New bone	50	41.32%
OB	9	7.44%



#### 4. DISCUSSION

Platelet-rich fibrin (PRF), a biomaterial composed of fibrin, encompasses beneficial components seen in a blood sample, including a substantial number of platelets and leukocyte

cytokines (Toffler M, Toscano N 2009 et.al.). Concentrated platelets are rich in many growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), insulin-like growth factor (IGF), epidermal growth factor (EGF), fibroblast growth factor

(FGF), and bone morphogenic protein (BMP). (Dohan DM 2006 et.al.). The growth factors mentioned above are of significant importance in the processes of hemostasis, angiogenesis, osteoblastic proliferation, and differentiation. This characteristic renders platelet-rich fibrin (PRF) a favorable option. The chemical composition and reduced thrombin levels of this substance create an ideal environment for the movement of endothelial cells and fibroblasts. (Prakash S, Thakur A. 2011), In the present study, it was noted that the application of platelet-rich fibrin (PRF) resulted in a reduction of inflammatory cells one week post-surgical treatment, as well as an increase in new bone formation.

Upon activation, platelets and leukocytes release cytokines that possess the capability to stimulate bone cells, hence playing a role in the regeneration of mineralized tissue. Additionally, it has been observed that macrophages present in platelet-rich fibrin (PRF) had the ability to enhance the process of bone production.

(Liu Y. 2017 et al.).

In a study conducted by Castillo et al., the efficacy of platelet-rich fibrin (PRF) in promoting bone repair following surgical extraction of the mandibular third molar was evaluated. The researchers employed a radiography approach identical to that employed in the current study. They observed a noteworthy increase in bone density after 8 weeks in the group receiving PRF treatment, with p-values less than 0.015.

However, the majority of research concur that platelet-rich fibrin (PRF) exerts a favorable impact on the healing of soft tissues and the regeneration of bones. Consequently, it can be employed as a standalone grafting material due to its ease of acquisition and absence of risks associated with alternative graft materials.

In our study, it was shown that i-PRF (injectable platelet-rich fibrin) was present as a foreign substance in the slides during the first week. Additionally, i-PRF demonstrated a reduction in inflammation. However, the development of new bone in the first weeks was greater in PRF (platelet-rich fibrin) compared to i-PRF. Furthermore, the PRF slides exhibited a higher occurrence of fibrous tissue formation.

According to Amiri et al. (2021), although there is a lack of substantial evidence about the efficacy of I-PRF in various regenerative

treatments, the authors assert that it is imperative to conduct rigorous randomized controlled trials (RCTs) to thoroughly examine the viability of I-PRF. In this context, scholars have recently proposed the potential utilization of I-PRF as a promising therapeutic intervention for the management of oral mucositis, wherein it exhibits superior efficacy in promoting soft tissue healing compared to hard tissue healing.

Currently, there is a need to further speed the development of liquid platelet concentrates while simultaneously avoiding the use of unnecessary anti-coagulants or other non-autogenous additions to their formulations which may prevent wound healing. Therefore, animal and clinical studies investigating the ability for i-PRF to improve wound healing and new bone formation for future clinical benefit remain necessary (Anitua E et al.)

## 5.CONCLUSION

PRF and i-PRF helps in the reduction of the inflammation response and increases collagen deposition and healing activity in the surgical site.

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