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PLATELET RICH FIBRIN EFFECT VERSUS HYALURONIC ACID EFFECT ON CRITICAL BONE DEFECT HEALING,(AN EXPERIMENTAL STUDY ON SHEEP.)

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ABSTRACT

Objective :The primary objectives of this study were to assess the influence of platelet-rich fibrin (PRF) and hyaluronic acid (HA) on the process of bone healing. This evaluation involved examining their effects on tissue response, inflammation, production of granulation tissue, as well as the existence of osteoid and the formation of new bone.

Background: The present study investigates the dynamic interaction between Platelet-rich fibrin (PRF) and Hyaluronic acid (HA) within this specific setting. Platelet-rich fibrin (PRF), which has a high concentration of growth factors, has been identified as a promising alternative to conventional bone grafts. In parallel, hyaluronic acid (HA) has been recognised for its significant contribution to tissue morphogenesis and the process of bone regeneration. This study examines the collective impacts and individual contributions of these factors on the acceleration of bone repair.

Methods: The study used a total of twelve adult male sheep, who were separated into four groups consisting of three sheep each. Surgical interventions were conducted, afterwards followed by postoperative assessments at certain time intervals (1, 8, 12, and 16 weeks). The histomorphometric evaluation was conducted to assess the course of bone healing.

Results: The experimental results indicate that Platelet-Rich Fibrin (PRF) has a notable cumulative impact on bone healing as time progresses. Likewise, HA demonstrated significant advancements over subsequent phases. Nevertheless, both the PRF and HA groups exhibited statistically significant differences when compared to the control group. Conclusion: The effect of PRF is mor than HA for bone healing enhancement, potentially reshaping clinical approaches.

***KEYWORDS**:* PRF, Hyaluronic Acid HA, Bone Healing, New bone, Osteogenesis.

1.INTRODUCTION

Encouraging fracture healing and, consequently, bone repair is the aim of clinical fracture treatment. Numerous treatments, including as local and systemic medicines, growth factors, bone-morphogenetic proteins, hyperbaric oxygen, hormones, and physical stimulation, have been shown to accelerate fracture healing in both clinical and experimental study.

Platelet-rich fibrin (PRF), which was originally developed by Choukroun et al (2001) in France, is a type of platelet concentrate that is commonly employed to expedite the healing process of both soft and hard tissues. The biomaterial in question comprises autologous leukocytes and platelet-rich fibrin (PRF), both of which possess angiogenic characteristics.

The cause of the latter phenomenon can be attributed to the intricate three-dimensional arrangement of the fibrin matrix. Within this matrix, various growth factors and cytokines are embedded simultaneously. These include platelet-derived growth factor, transforming growth factor-b1, insulin-like growth factor, and vascular endothelial growth factor. The Platelet-Rich Fibrin (PRF) is believed to facilitate the progression of healing and regenerative mechanisms by assuming a pivotal function in the shift from inflammation to wound repair in the context of osteogenesis.

Hyaluronic acid (HA) is a glycosaminoglycan that is naturally found in high quantities in the early fracture callus, lacunae surrounding hypertrophic chondrocytes in the growth plate, and the cytoplasm of osteoprogenitor cells. It is hydrophilic and nonimmunogenic in nature. The hyaluronic acid (HA) possesses osteoconductive

characteristics that facilitate bone formation. It also expedites the process of bone regeneration by promoting chemotaxis, proliferation, and the subsequent differentiation of mesenchymal cells.

Numerous research have provided empirical evidence about the beneficial impacts of platelet-rich fibrin (PRF) and hyaluronic acid (HA) on the process of bone regeneration. Nonetheless, the number of comparative studies conducted in this area remains limited.

The aim of study was to determine the effect of PRF and HA on bone healing defect and new bone formation were evaluated histomorphometrically.

2.MATERIAL AND METHOD

This study is carried out on 12 adult local healthy male sheep, their age ranged between (6 to 8) months with body weight ranged between (28 to 34) kg. The sheep 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. All experiments on animals were approved by the research ethics committee follow the council for international organization of medical science ethical code for animal preparation (IOM, 1985). sheep are subjected to a thorough clinical examination and complete blood examination to ascertain their health status.

Proper incision is done (about 3 cm in length and about 1 cm below and parallel to the mandible). Blunt dissection was followed to expose the lateral surface of the mandible by the blunt dissector, then the mucoperiosteal elevator was used to reflect the periosteum. Then 3 small cavities of 2 mm in depth and 3 mm in diameter was drilled into right side of the sheep mandible with straight handpiece and standard bur (trephine bur) under copious irrigation with normal saline, a space of about (1cm) was left between the holes.

The 1rst cavity filled with PRF and the 2nd one by HA the 3rd one left without foreign body as a control. sheep subdivided into 4 groups post operatively according to sacrifice to investigate the phases of bone healing (1-8-12-16) weeks.

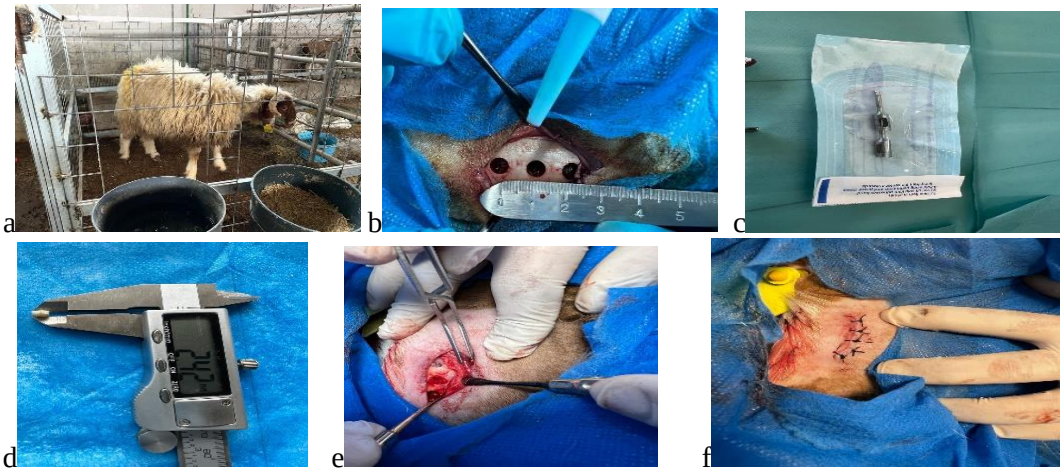


Fig.(1):- surgical procedure, (a) The sheep are housed in animal indoors, (b)1cm between each holes,(c)trephine bur for bone defect,(d) 2-3mm diameter of bone removed(e) insertion of PRF, HA in the holes,(f)suturing the flap.

PRF Preparation:

In order to obtain Platelet-Rich Fibrin (PRF), it is necessary to expeditiously collect a sufficient volume of blood into test tubes without the presence of an anticoagulant. Subsequently, the collected blood should be promptly subjected to centrifugation. Blood can be subjected to centrifugation using a tabletop centrifuge for a minimum duration of 10 minutes at a rotational speed of 3000 revolutions per minute. The resultant product comprises three distinct layers: the uppermost layer consisting of platelet poor plasma, the middle layer composed of PRF clot, and the bottom layer consisting of 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 process, the layer of red blood cells (RBCs) that is attached to the platelet-rich fibrin (PRF) clot can be carefully excised using a sterilised pair of scissors. According to Mufti and Sonam (2017)

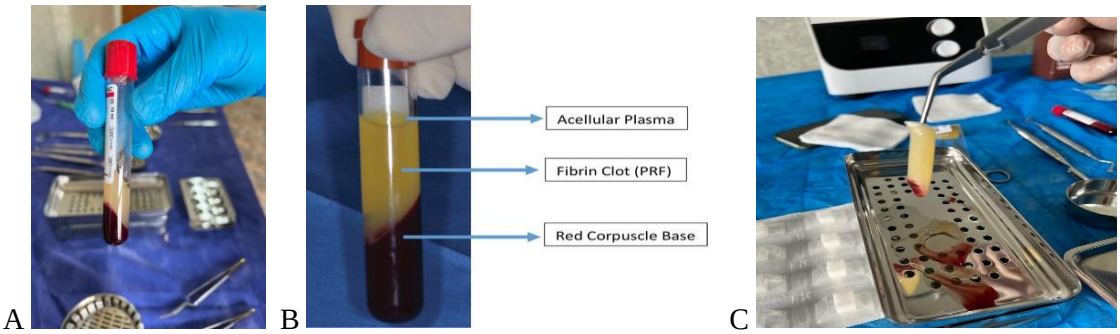


Fig.(2):- PRF preparation (a) PRF in anticoagulant tube (b) PRF with RBC layer (c) PRF ready for insertion.

HA preparation:

The application of hyaluronic acid (specifically, the Hyaloss™ matrix) has been shown to facilitate an improved and expedited healing process, as supported by histological assessments. In our study, we utilised a 2% vial of Hyaluronic acid in gel form, which was obtained from CMMC in Spain. The application procedure employed in our study was consistent with the methodology outlined by Muzaffer in 2006.

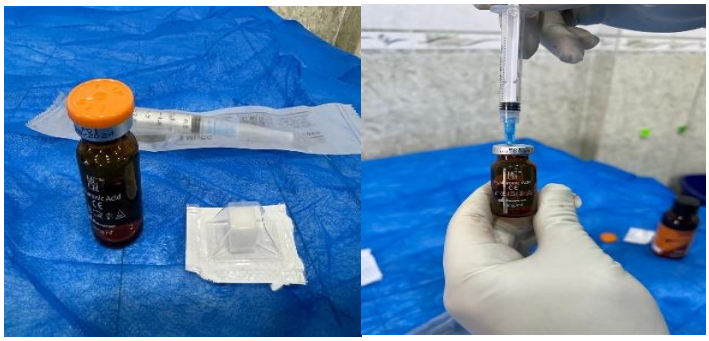


Fig.(3): -Hyaluronic acid 2% vial in gel form ready origin (CMMC -Spain)

3.RESULTS

3.1Clinical Results: In total, 36 defects in 12 sheep 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.

3.2Histological Findings:

Once the slides have been prepared, they are ready for histomorphometric analysis. The application of hematoxylin eosin staining was utilized on the slides in order to see and highlight the characteristics of the cytoplasm, nucleus, and extracellular matrix.

This study revealed an inflammatory response and proliferation of osteocytes at the time points of 1 week and 16 weeks. Distinct types of cells were delineated using various colored circles in order to distinguish the process of new bone creation.

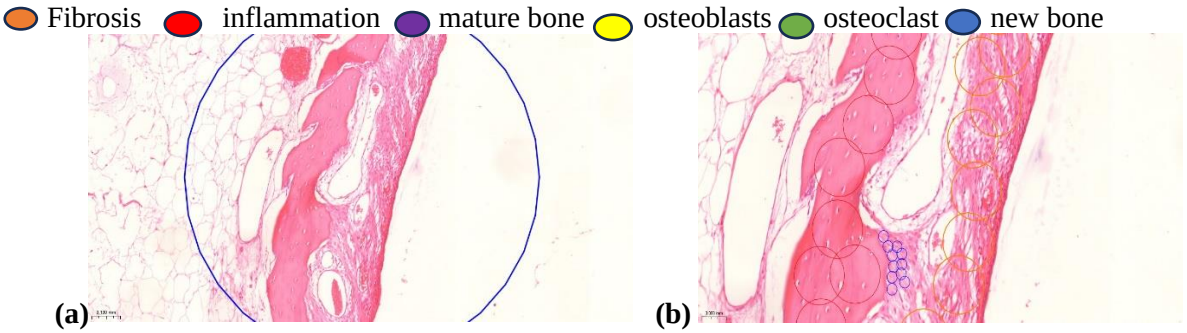
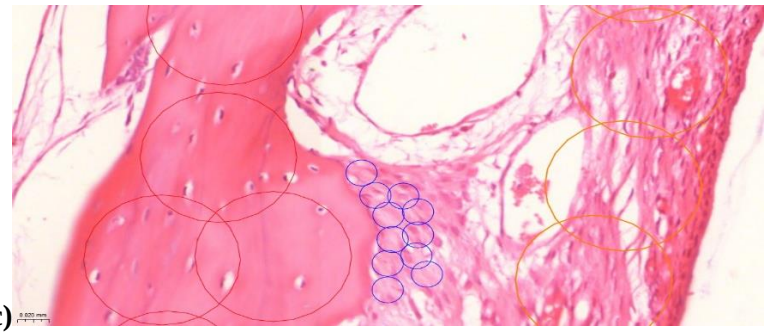


Fig. : (a)10x (b)20x first weeks using PRF after surgery shows inflammatory tissue with fibrous and formation of new bone .

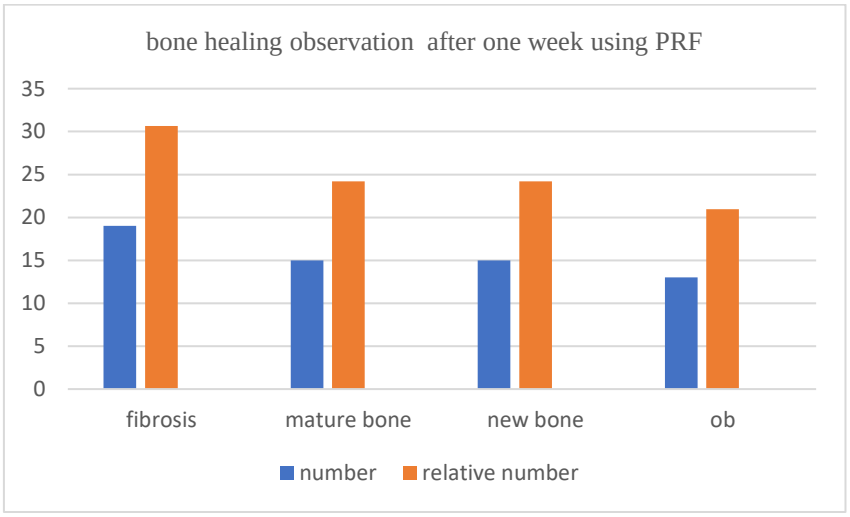


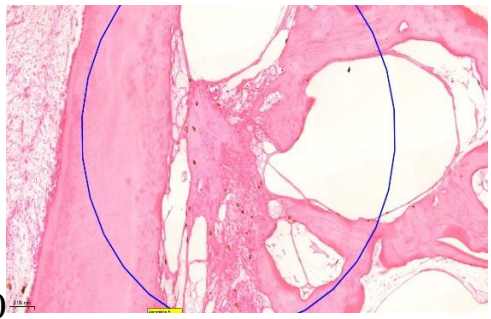
(c)

Fig.: (c) 40x zoomed near the inflammatory tissue and new bone after one week of PRF incertion, the inflammation is more obvious and new bone strains are formed near this tissue.

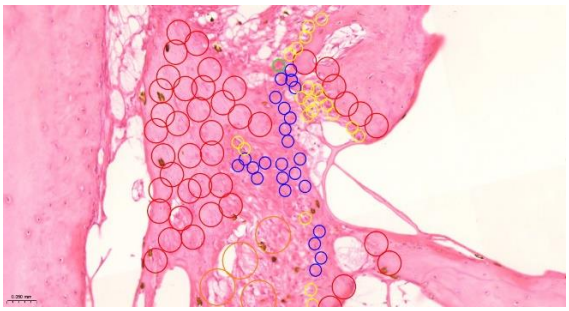
Fibrosis inflammation mature bone osteoblasts osteoclast new bone

Category	Number	Relative number
Fibrosis	19	30.65%
Mature bone	15	24.19%
New bone	15	24.19%
Ob	13	20.97%



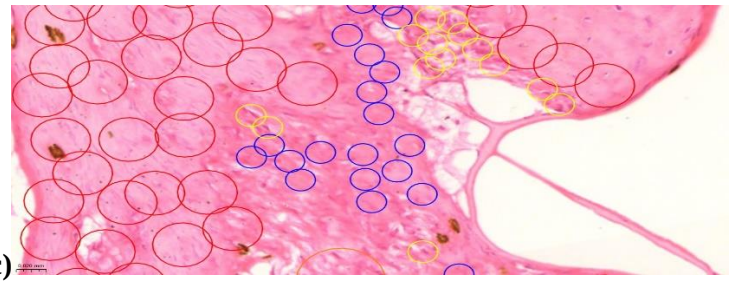


(a)



(b)

Fig.: (a) 10x (b) 20x first week using HA shows more inflammatory response with formation of new bone and osteoclastic activity.

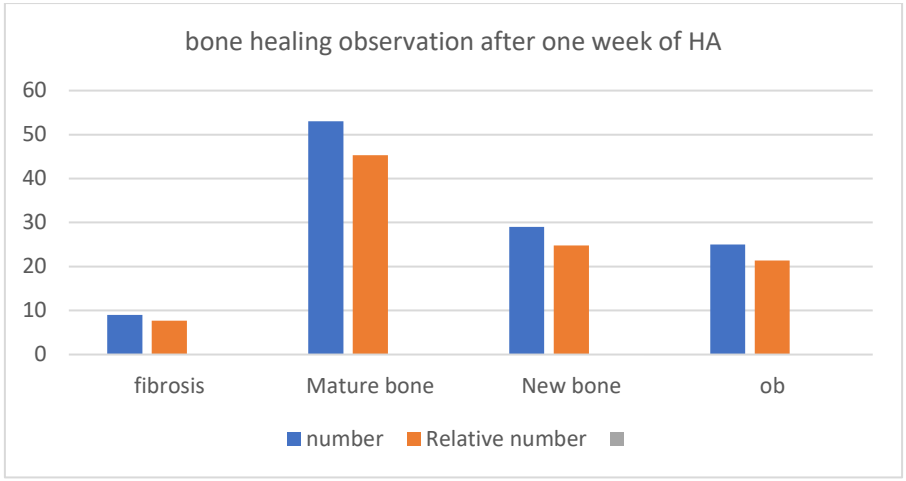


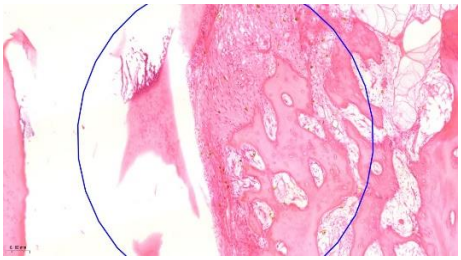
(c)

Fig.: (c) 40x one week inflammatory response with osteoclastic activity with new bone using HA .

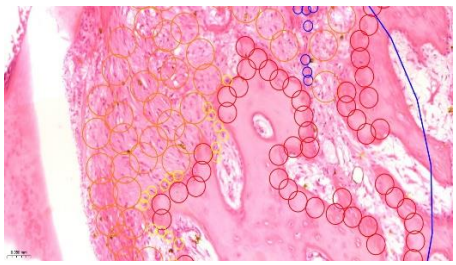
Fibrosis inflammation mature bone osteoblasts osteoclast new bone

Category	Number	Relative number
Fibrosis	9	7.69%
Mature bone	53	45.30%
New bone	29	24.79%
Ob	25	21.37%
Osteoclasts	1	0.85%





(a)



(b)

Fig.: (a) 10x shows inflammation in controlled group of bone healing after one week. (b) 20 x inflammation and fibrosis with osteoclastic activity more obvious in first week

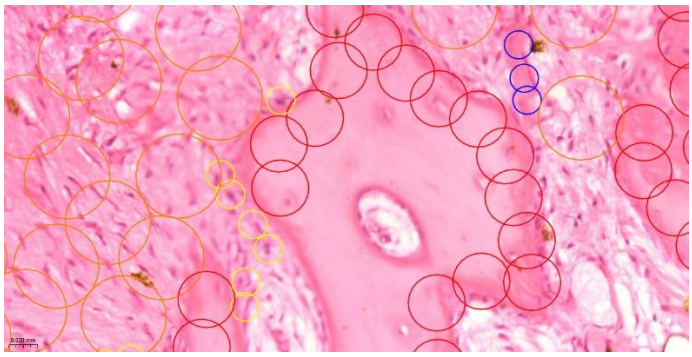
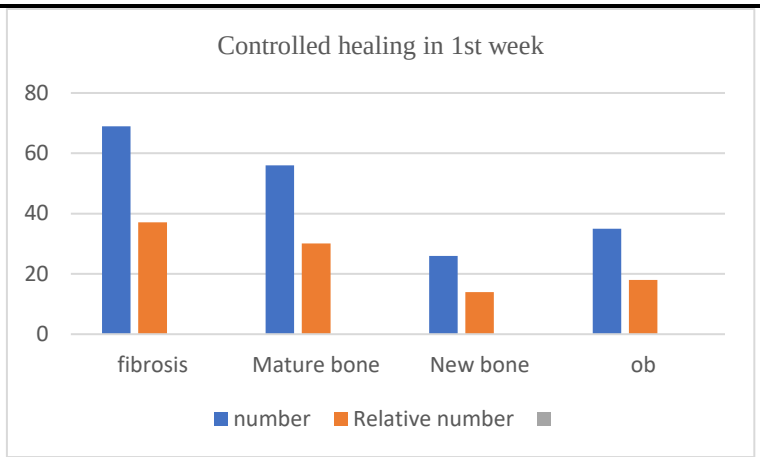
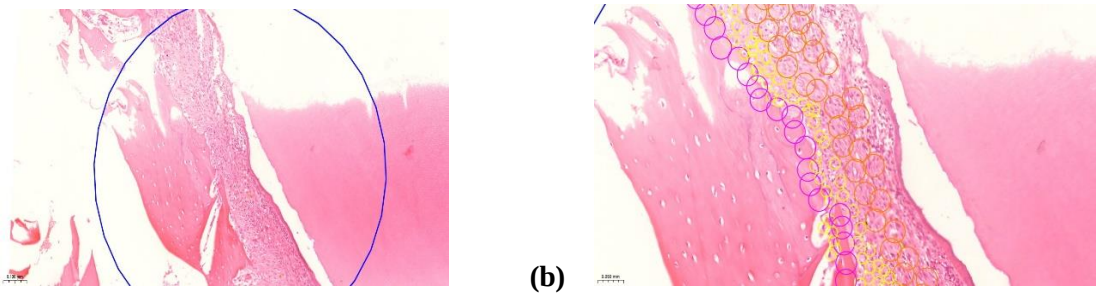


Fig.: (c) 40x first week shows the inflammation more obvious

Fibrosis inflammation mature bone osteoblasts osteoclast new bone

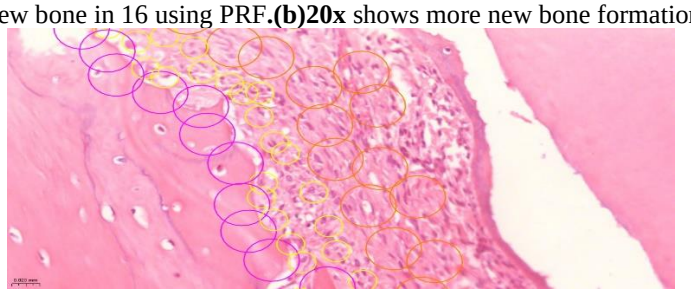
Category	Number	Relative number
Fibrosis	69	37.1%
Mature bone	56	30.11%
New bone	26	13.98%
Ob	35	18%
Osteoclasts	5	0.85%





(a) **(b)**

Fig.:(a)10x formation of new bone in 16 using PRF.**(b)20x** shows more new bone formation in 16 weeks of PRF

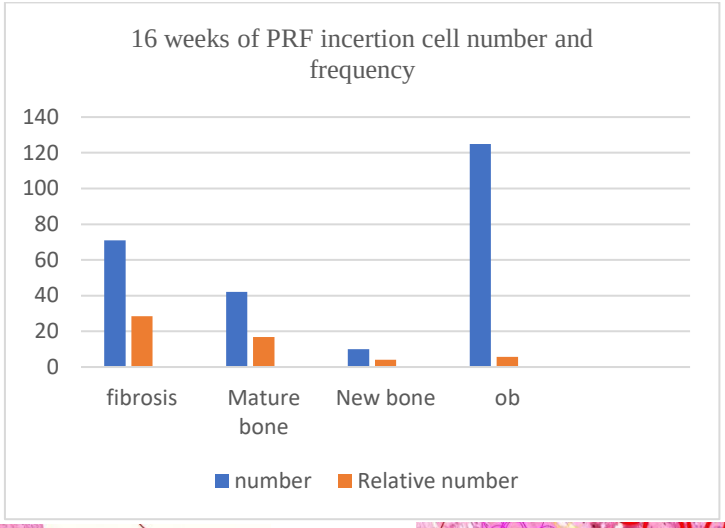


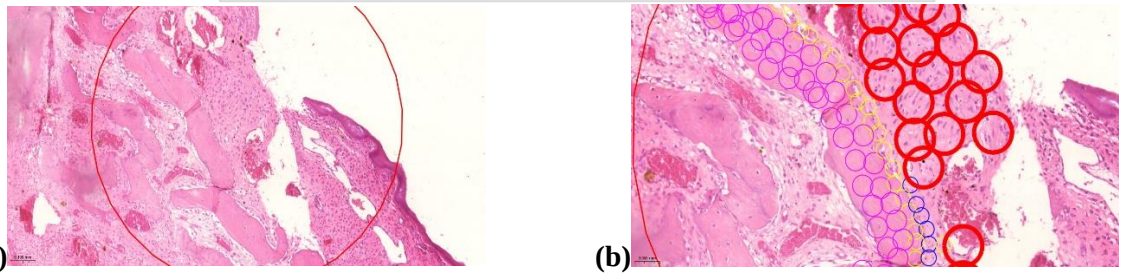
(c)

Fig.:(c) 40x shows osteoblast, new bone, mature bone in 16 weeks of PRF insertion

Fibrosis inflammation mature bone osteoblasts osteoclast new bone

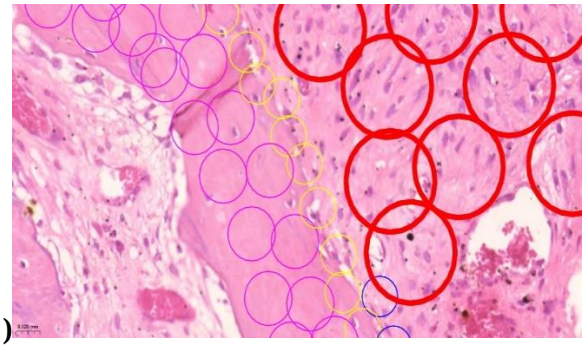
Category	Number	Relative number
Fibrosis	71	28.51%
Mature bone	42	16.87%
New bone	10	4.02%
Ob	125	5.60%





(a) **(b)**

Fig.:(a)10x formation of mature bone in 16 weeks using HA **(b) 20x** shows mor bone formation in 16 weeks using HA



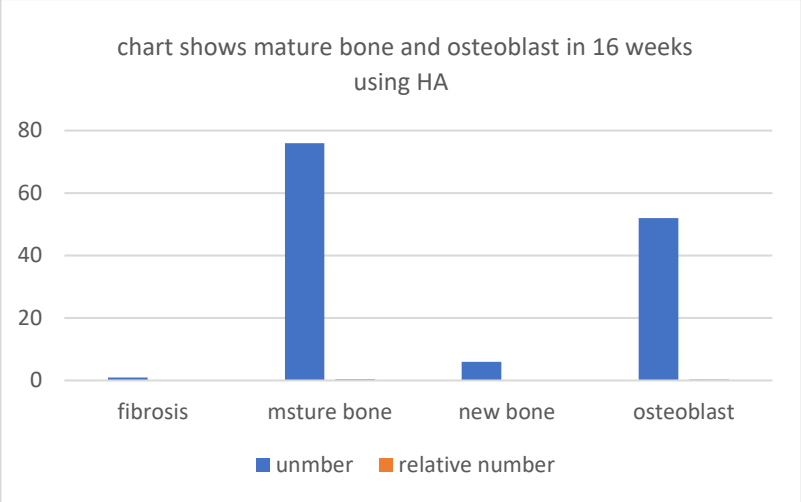
(c)

Fig.: (c)40x shows osteoblast and mature bone and inflammatory cells in 16 weeks using HA

Fibrosis inflammation mature bone osteoblasts steoclast ew bone

Category	Number	Relative number
Fibrosis	1	0.60%
Mature bone	76	45.51%
New bone	6	3.59%
Ob	52	31.14%

Chart of HA in 16 weeks



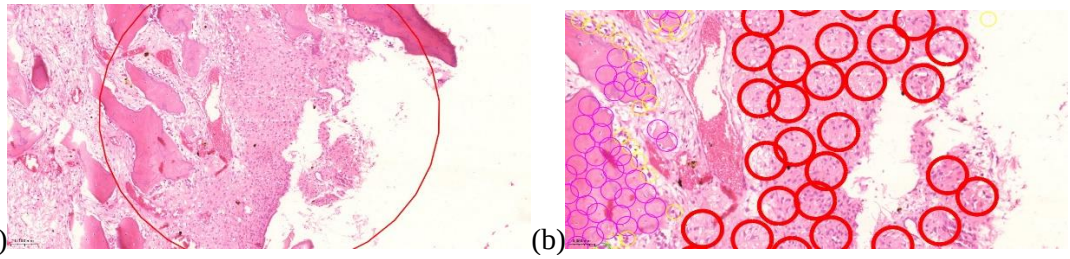


Fig.:(a)10x controlled healing in 16 weeks **(b) 20x** controlled healing shows mor inflammatory cells in 16 weeks

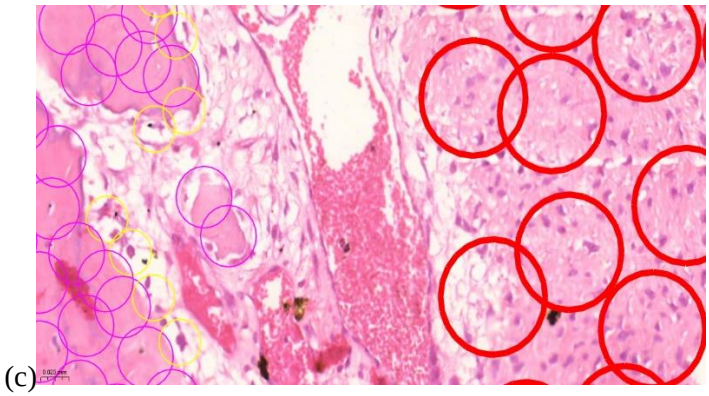
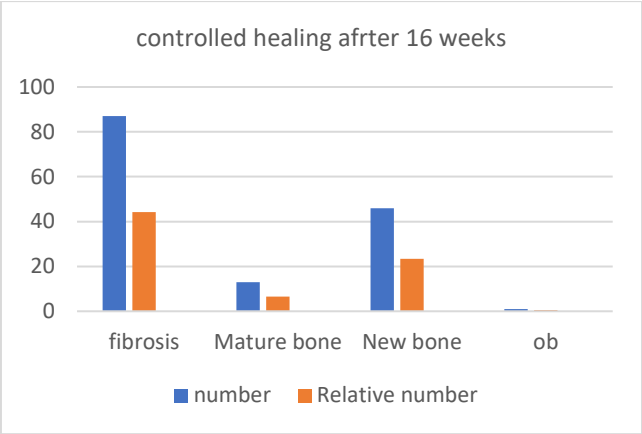


Fig.:(c) 40x control healing in 16 weeks

Fibrosis inflammation mature bone osteoblasts osteoclast new bone

Category	Number	Relative number
Fibrosis	87	44.16%
Mature bone	13	6.60%
New bone	46	23.35%
Ob	1	0.51%

16 weeks controlled healing



4.DISCUSSION

Platelet-rich fibrin (PRF), classified as a fibrin biomaterial, contains beneficial components found in a blood sample, including a significant number of platelets and leukocyte cytokines (Toffler M, Toscano N 2009 et.)

According to Dohan et al. (2006), concentrated platelets are rich in various 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, and bone morphogenic protein. The growth factors mentioned above have a significant impact on processes such as hemostasis, angiogenesis, and osteoblastic proliferation and differentiation. This characteristic of platelet-rich fibrin (PRF) provides it with distinct advantages. The chemical composition and reduced thrombin levels of this substance create an ideal environment for the movement of endothelial cells and fibroblasts. Prakash and Thakur (2011) conducted a study.

In the present study, it was discovered 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 the formation of new bone tissue.

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 promote bone formation, as demonstrated by (Liu et al. (2017).

In a study conducted by Castillo GF 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 the same radiography methodology as the current study and observed a noteworthy increase in bone density after 8 weeks in the group receiving PRF treatment. The statistical analysis revealed p values of less than 0.015, indicating a meaningful outcome.

Nevertheless, a consensus among the majority of research studies indicates that Platelet-Rich Fibrin (PRF) has a beneficial effect on the process of soft tissue healing and bone regeneration. Therefore, it can be utilized as an independent grafting material owing to its convenient accessibility and absence of associated dangers observed in other graft materials.

Hyaluronic Acid (HA) enhances the healing process by its ability to stabilise matrices, facilitate cell infiltration and migration, and regulate fibrin breakdown. The hydrophilic nature of the substance enables it to attract and hold water, creating a moist environment that is crucial for processes such as cell migration, tissue repair, and general healing (Vasvani et al., 2020). In addition, the anti-inflammatory characteristics of HA play a role in creating a favorable setting for bone healing by mitigating the inflammatory phase (Schlundt et al., 2015).

Likewise, the impact of HA on bone density in the first stages of the healing process may be subject to the influence of the necessary time required for facilitating repair. Various factors, such as concentration, formulation, and treatment methods, may potentially play a role in the absence of statistically significant variations in bone density during the early stages of recovery. The properties of hyaluronic acid (HA), namely in its role in facilitating cell migration, proliferation, and tissue remodeling,

may exhibit greater prominence throughout the later phases of the healing process (Aslan et al., 2006; Park et al., 2016; Zhai et al., 2020).

Hyaluronic acid is an osteoconductive substance that promotes osteogenesis and expedites the healing process within intra bone defects, surpassing the pace of healing observed in the normal physiological process. According to Radhi and Al-Ghaban (2015).

5.CONCLUSION

This work has provided evidence for the beneficial impacts of platelet-rich fibrin (PRF) and hyaluronic acid (HA) on bone regeneration. However, limited research has been conducted to compare the relative efficacy of PRF and HA in promoting new bone formation.

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