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ENHANCING BONE HEALING WITH AUTOGENOUS PRP AND HYALURONIC ACID: AN EXPERIMENTAL STUDY

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ABSTRACT

Objective: The aims of this study was to unveil the accelerated bone healing potential of PRP and HA, either separately or in a mixture. By evaluating their impact on tissue response, inflammation, granulation tissue formation, and osteoid presence.

Background. This experimental study explores the dynamic interplay of Platelet-rich plasma (PRP) and Hyaluronic acid (HA) in this context. PRP, rich in growth factors, has emerged as a potential substitute for traditional bone grafts, while HA plays a crucial role in tissue morphogenesis and bone regeneration. This research investigates their combined effects and individual contributions to bone healing acceleration.

Methods: Twenty-five adult male rabbits were introduced in the study and divided the subjects into five groups (each group 5 rabbits). Surgical procedures were performed, followed by postoperative assessments at intervals (days 3, 7, 14, 21, and 28). Bone healing progression was evaluated using a histopathological and computed tomography CT scans.

Results: PRP alone demonstrated significant a cumulative effect over time, enhancing bone density. Similarly, HA exhibited noteworthy improvements at later stages. However, their combination showed significant differences compared to the control group, highlighting a potential synergistic effect on bone healing at days 14, 21, and 28.

Conclusion: The combination between PRP and HA holds promise for bone healing enhancement, potentially reshaping clinical approaches.

KEYWORDS: Autogenous PRP, Hyaluronic Acid, Bone Healing, Oral Surgery, Osteogenesis.

1-INTRODUCTION

Bone histology involves cellular components like osteoblasts, osteocytes, osteoclasts, and osteogenic precursor cells. Bone morphogenetic proteins (BMPs) play vital roles in bone growth and repair. Understanding fracture healing at the molecular level has identified key molecules for enhancing bone repair (Van et al., 2021; Dimitriou et al., 2012).

Bone healing can be categorized into primary and secondary healing. Primary healing occurs with stable fractures and resembles normal bone remodeling . Secondary healing, more common, involves inflammation, repair, and remodeling phases (Maruyama et al., 2020).

Platelet-rich plasma (PRP) is a concentrated form of platelets derived from an individual's own blood, containing growth factors that aid in tissue healing and regeneration (Marx, 2001). PRP has various growth factors such as PDGF, TGF-β, VEGF, EGF, IGF-1, and FGF, which play a role in accelerate bone healing, promoting tissue regeneration (Moshiri & Oryan, 2013; Marques et al., 2015).

PRP is prepared through centrifugation, either by the open technique with multiple spins or closed commercial kits (Alves & Grimalt, 2018). Activation of PRP with substances like thrombin and calcium chloride releases growth factors for tissue healing (Anitua et al., 2011). PRP has shown promising effects on bone healing and regeneration in various dental and orthopedic applications (Verma et al., 2019; Zhang et al., 2018).

Hyaluronic acid (HA) is a polysaccharide that belongs to the glycosaminoglycan family and consists of a basic unit of two sugars, glucuronic acid, and N-acetyl-glucosamine (Neuman et al., 2015). HA is a key element in various tissues, such as joints, cartilage, and skin, and it plays essential roles in tissue repair and regeneration (Casale et al., 2016).

HA is abundantly present in the extracellular

matrix of the skin, where it maintains the rheological, hygroscopic, and viscoelastic properties of the tissue (Gupta et al., 2019). It is involved in tissue repair processes, but the specific mechanisms by which it influences tissue repair are not fully understood (Ghatak et al., 2015).

Research has shown that HA can modulate the inflammatory, proliferative, and remodeling phases of the tissue healing process, and its effects can be either anti-inflammatory or pro-inflammatory, depending on its molecular weight (Litwiniuk et al., 2016).

HA also plays a crucial role in bone healing and regeneration. It acts as a temporary structure in the initial stages of bone healing, facilitating the movement of nutrients and waste products within the wound site (Holmes et al., 1988). Studies have shown that HA carriers can enhance osteogenesis and mineralization, promoting bone regeneration (Zahi et al., 2020).

The combination of HA with platelet-rich plasma (PRP) has shown promising results in various clinical settings. This combination therapy has been found to have significant advantages, including improved pain relief, reduced immune responses, enhanced angiogenesis, and better histological parameters in healing (Yu et al., 2018). The combined use of HA and PRP has demonstrated effectiveness in treating bone and joints inflammations, leading to significant improvements in pain relief and functional outcomes (Gilat et al., 2021).

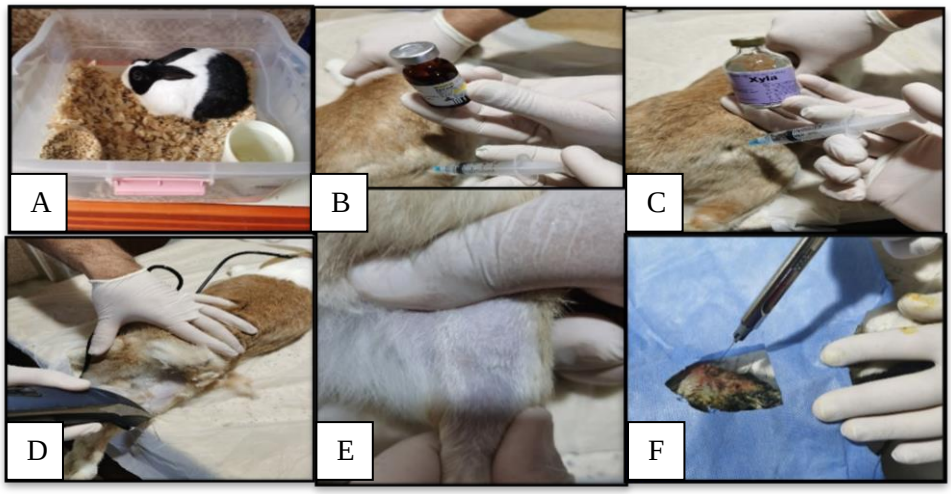


Fig.(1) :-Pre-operative Preparation of:(A) A Rabbit in Cage, (B)Injection of ketamine, (C)Injection of Xylazine, (D)shaving surgical area, (E)Identification of Saphenous Vien, (F)Injection of LA

2-MARTIALS AND METHODS

The surgical part of the study was done at Biology Department, College of Science, University of Duhok, Begins from December, 2021 to, July 2022. 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).

This study was carried out on local; twenty-five adult male rabbits aged (8-10 months) of weighting (2000-2500 g) was used as experimental animal. During the entire period of the experiment the rabbits were kept in cages and feed three times daily with greenery diet and tap water and general health continuously monitored by veterinary medicine Fig. (1). The rabbits will be randomly classified into five groups, each group of five rabbits as follows: Group I: received the surgical procedure and sacrificed at day 3 post operatively, Group II: received the surgical procedure and sacrificed at day 7 post operatively, Group III: received the surgical procedure and sacrificed at day 14 post operatively, Group IV: received the surgical procedure and sacrificed at day 21 post operatively and Group V: received the surgical procedure and sacrificed at day 28 post operatively.

MATERIALS USED IN SURGERY

The martials that were used in the surgery as shown in Fig. (3) they are:

1. Hyaluronic acid 2% vial in gel form ready

origin (CMMC -Spain)

2. PRP which is autogenously prepared

Autogenously PRP Preparation:

Aspiration about 2-3 ml of blood was drawn from the Saphenous Vein which is anatomically located across the lateral aspect of tibia Fig. (2) this done with 23-gauge needle syringe. Sample was transferred

Fig.(2): -Steps of preparation of Autogenous PRP:(A) palpation and localization of Saphenous Vien, (B)Blood Aspiration, (C)Blood in Anticoagulant tube, (D)Centrifugations device, (E)Blood at first centrifugation, (F)Blood at2nd centrifugation to sterile tubes containing sodium citrate (anticoagulant) The PRP prepared by double centrifugation protocol (Pazzini et al., 2016). The blood was first centrifuged at 1600 rpm / 10 mint, resulting in the separation of plasma containing platelets and leucocytes from red blood cells and then centrifuged plasma was drawn off from top and centrifugated for second round at 2660 rpm for 10 minutes Fig. (2). It was resulted in two parts: on the top, consisting of platelet-Rich plasma (PRP) and at the bottom of the tube red blood cells. Part of the PRP was discarded so that only 1ml remained in the tube along with platelet button. This material was gently agitated to promote platelet resuspension result at the time off application the PRP was combined with equal volume of sterile solution of 10% $Cacl_2$ activation inhibitor that allow plasma to coagulate and cause activation of platelet to regenerate the growth factor in in the production of platelet-rich plasma (PRP) Fig. (2).

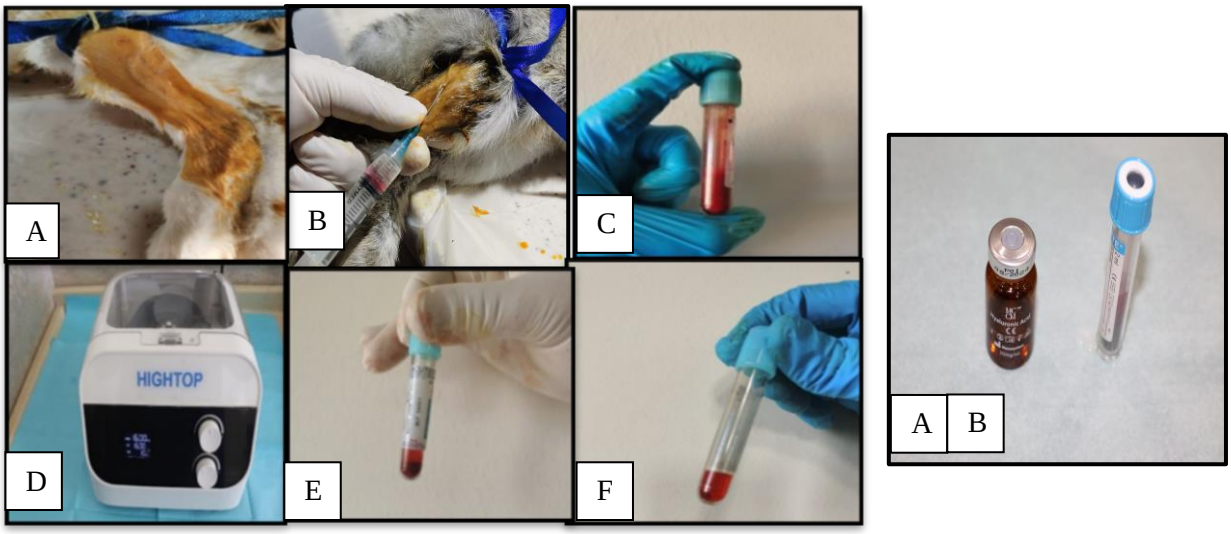


Fig. (2):- Steps of preparation of Autogenous PRP**:(A)** palpation and localization of Saphenous Vien**, (B)**Blood Aspiration**, (C)**Blood in Anticoagulant tube, **(D)**Centrifugations device**, (E)**Blood at first centrifugation, **(F)**Blood at2nd centrifugation

Fig.(3):- *Martials that were used in Research:* ***A.*** *HA,* ***B****. PRP*

Surgical procedures

pre-operative animals' preparation:

The surgical sites (Tibias) were cleaned and washed by soap and tap water and dried by clean towel a day before the surgical procedure.

Induction of Anesthesia

The animals were anaesthetized intra-muscularly with (10 % 50mg/kg) Ketamine Fig. (1) (Dar-Aldewa-Jordan) then followed by intra-muscularly (20mg 2 mg/kg). of Xylazine (Interchemie- Holland) Fig. (1) for muscle relaxation. Complete anesthesia was observed approximately within 5 to 10 minutes. During the waiting period till the rabbits were fully anesthetized then the hair over the surgical sites was shaved by electrical hair shaver Fig. (1).

Surgical Operation

The animal was placed in lateral position during procedure, then L.A of 2 % of lidocaine Hcl with epinephrine1:80:0000 (Normon-Ispain) were administrated by infiltration to provide hemostasis at surgical site Fig. (1).

After disinfection the surgical site with povidone iodine solution 10 % a 5cm longitudinal incision was made along right tibia begins about 1 cm below femur articulation with

tibia, then skin and subcutaneous tissue was dissected with associated muscles to expose the underlying bone. The two monocortical holes were created by using surgical low speed handpiece at 1500 RPM with copious irrigation by normal saline and continuous suctioning to provide cooling with trephine bur with 2X2 mm in width and depth. The first hole Immediately after the tibia articulate with femur and left empty considered as control, the second hole were made about 1 cm from the first hole and implanted with autogenously PRP previously prepared with sponge as carrier. Then the created incision was closed in layers and sutured by Silk 3/0 suture simple entrapped matter suture(fig4). The same procedure repeated in the left Tibia with the first hole Immediately after the tibia articulate with femur and Implanted with HA (2 % CMMC-Spain) and the second hole were made about 1 cm from the first hole and Implanted with PRP autogenously prepared Mixed with HA (2 % CMMC-Spain) mixing was done on glass stab. Then the created incision was closed in layers and sutured by Silk 3/0 suture simple entrapped matter suture.

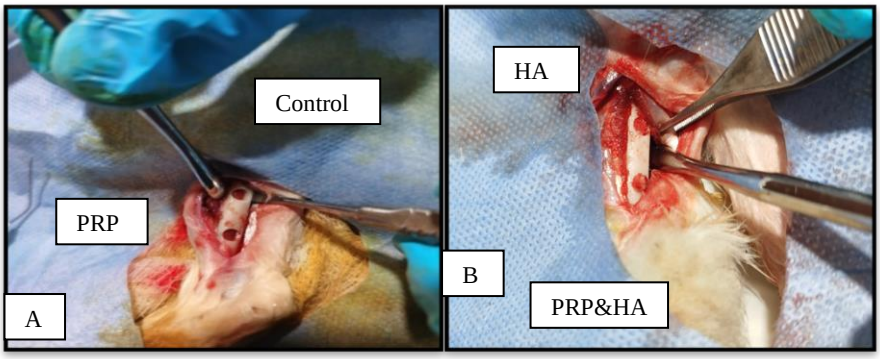


Fig.(4):- surgical operation:(A) Right Tibia Surgery, (B)Lift Tibia Surgery

Post-operation follow-up

Post-Operative Care After completing the surgical procedure the experimented animals were immediately injected with gentamicin sulfate ampule solution 80mg. intramuscularly and gentamycin ointment externally over the surgical site of both tibia after finishing the surgical procedure daily till day 7 post operatively for all groups. The rabbits were kept under observation till they completely recovered from anesthesia. Post-operative monitoring of the operated animals during first twenty-four hours including observation of their feeding and physical activity.

Specimen Collection and Preparation

After completing the decided healing period, the animals were scarified and the tibias were dissected and kept in 10% formalin for fixation, and subsequent histopathological slide preparation.

3-THE RESULTS

The results of the this study aimed on evaluating the effects of different treatments on bone density, granulation tissue formation, inflammatory response, and new bone formation in rabbit tibia bone defects. The study used CT scans to measure bone density and histopathological analysis to assess tissue responses.

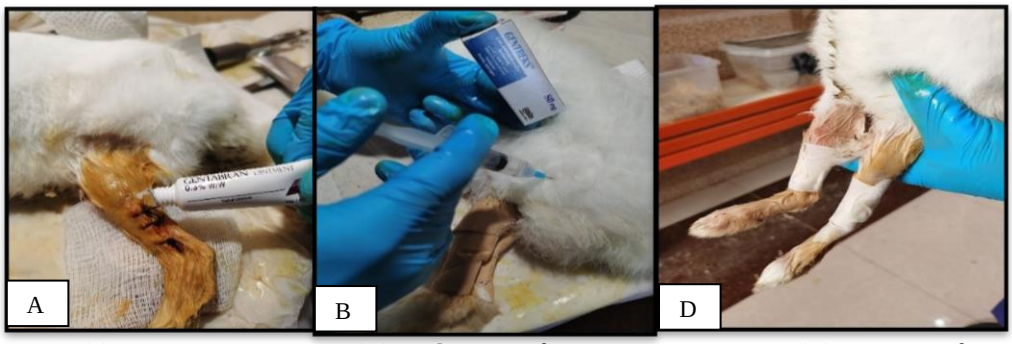


Fig. (5): -Post operation care: (A)Application of gentamycin Ointment, (B) Injection of Gentamycin, (C) Wound dressing

Bone Density Analysis (CT scans):

CT scans were used to measure bone density using Hounsfield units (HU) as in table (1). Bone density was evaluated at different time intervals (days 3, 7, 14, 21, and 28) for different treatment groups: Control, PRP, HA, and PRP & HA.The results indicated that PRP, HA, and PRP & HA treatments led to significant increases in bone density compared to the control group at various time points, with PRP & HA showing the highest impact. The statistical analysis confirmed the significant differences in bone density between treatment groups.

Table (1):- Mean of CT Results of Bone Dentisty

	Day 3 Mean ± SD	**Day 7 Mean ± SD**	**Day 14 Mean ± SD**	**Day 21 Mean ± SD**	**Day 28 Mean ± SD**
Control	61.6 ± 4.04	111.6 ± 7.73	159.8 ± 10.57	219.6 ± 18.35	304.4 ± 11.8
PRP	71.2 ± 5.67	137.6 ± 7.02	171.6 ± 7.6	249.1± 19.40	323.4 ± 12.8
HA	88.6 ± 6.11	139.6 ± 5.59	156.2 ± 10.85	250.9 ± 19.38	324.2 ± 10.6
PRP & HA	93.8 ± 7.6	146 ± 4.06	180.6 ± 11.37	290.6 ± 19.35	364.4 ± 11.2

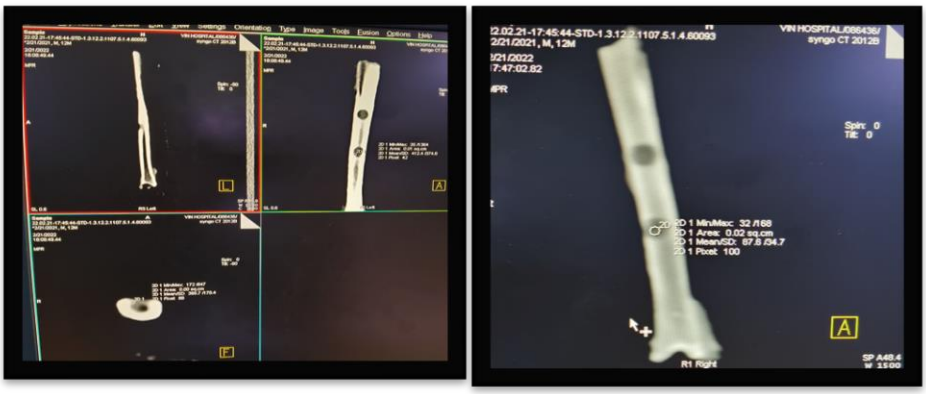


Fig.(6):- CT scans Analysis

Histopathological Analysis:

The study evaluated granulation tissue formation, inflammation responses, and new bone formation through histopathological analysis. Bone healing assessment was histopathology done by using a grading system to evaluate the healing process of the bone defects. The grading system was previously used by several researchers (Kim et al., 2004; Maeda et al. 2007; Abrahem and Al-Soudani, 2012; Elgendy et al., 2013). This grading system was based on the degree of tissue response in relation to inflammation, granulation tissue formation, and the presence of osteoid tissue. Each criterion was assigned four grades, ranging from nil to severe. (The histopathological slides were examined by the two specialists and the researcher in a blinded manner using a light microscope.

Table (2):- Histopathological Criteria for Grading

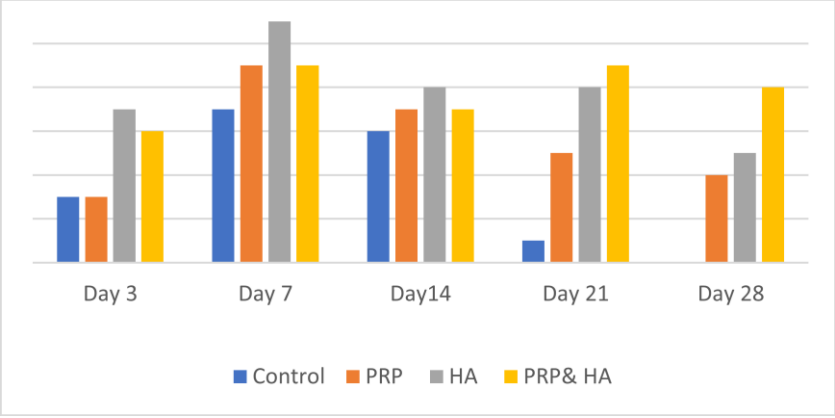
Grading formation of granulation tissue		**Grading of inflammation tissue**		**Grading of new bone formation**	
Grade	Histopathology Criteria	Grade	Histopathology Criteria	Grade	Histopathology Criteria
0 / Non	No granulation tissue	0 / Non	No inflammation	0 / Non	No new bone formation
I / mild	Granulation tissue Less than 50	I / mild	few lymphocytes or macrophages	I / mild	New bone formation at periphery
II/moderates	Granulation tissue between 50-75	II/moderates	several lymphocytes and macrophage with few neutrophils or FB	II/moderate	New bone formation at center
III / severe	Granulation tissue More than 75	III / severe	large number of all	III / severe	New bone formation at periphery and center

Table (3): -Results of Histopathological Analysis

Granulation Results of Histopathological Analysis					
	Day 3	Day 7	Day14	Day 21	Day 28
Control	**0, 0, I, I, I**	**I, I, I, II, II**	**I, I, I, I, II**	**0, 0, 0, 0, I**	**0, 0, 0, 0, 0**
PRP	**0, 0, I, I, I**	**I, II, II, II, II**	**I, I, I, II, II**	**0, I, I, I, II**	**0, I, I, I, I**
HA	**I, I, I, II, II**	**I, II, II, III, III**	**I, I, II, II, II**	**I, I, II, II, II**	**I, I, I, I,I**
PRP& HA	**I, I, I, I, II**	**I, II, II, II, II**	**I, I, I, II, II**	**I, II, II, II, II**	**I, I, II, II, II**
Inflammatory Results of Histopathological Analysis					
	Day3	Day7	Day14	Day21	Day27
control	**I, I, I, I, II**	**II, II, II, III, III**	**I, I, I, II, II**	**I, I, I, I, II**	**0, 0, 0, 0, I**
PRP	**I, I, I, II, II**	**II, II, II, III, III**	**I, I, I, II, II**	**I, I, I, I, I**	**0, 0, 0, 0, 0**
HA	**I, I, I, II, II**	**II, II, II, III, III**	**I, I, I, II, II**	**I, I, I, I, I**	**0, I, I, I, I**
PRP & HA	**I, II, II, II, II**	**II, II, III, III, III**	**II, II, II, III, III**	**0, I, I, I, I**	**I, I, I, I, I**
Bone formation Results of histopathological Analysis					
	Day3	Day7	Day14	Day21	Day28
control	**0, 0, 0, 0, 0**	**0, 0, 0, 0, 0**	**0, 0, 0, 0, 0**	**0, 0, 0, 0, 0**	**I, I, I, I, II**
PRP	**0, 0, 0, 0, 0**	**0, 0, 0, 0, 0**	**0, 0, 0, 0, 0**	**0, I, I, I, I**	**I, I, I, II, II**
HA	**0, 0, 0, 0, 0**	**0, 0, 0, 0, 0**	**0, I, I, I, I**	**I, I, I, II, II**	**I, I, I, II, II**
PRP & HA	**0, 0, 0, 0, 0**	**0, 0, 0, 0, 0**	**I, I, I, II, II**	**II, II, II, III, III**	**III, III, III, III, III**

Granulation tissue formation:

Histopathological analysis of granulation tissue formation at different time points (day 3, 7, 14, 21, and 28) reveals insights into bone defect healing. Grading from non to severe, the PRP group showed early mild healing on day 3, progressing to moderate on day 7. HA and PRP & HA groups also demonstrated mild healing on day 3, evolving to moderate by day 7. By day 14, all groups exhibited moderate healing.



Chart(1):- Granulation Formation analysis

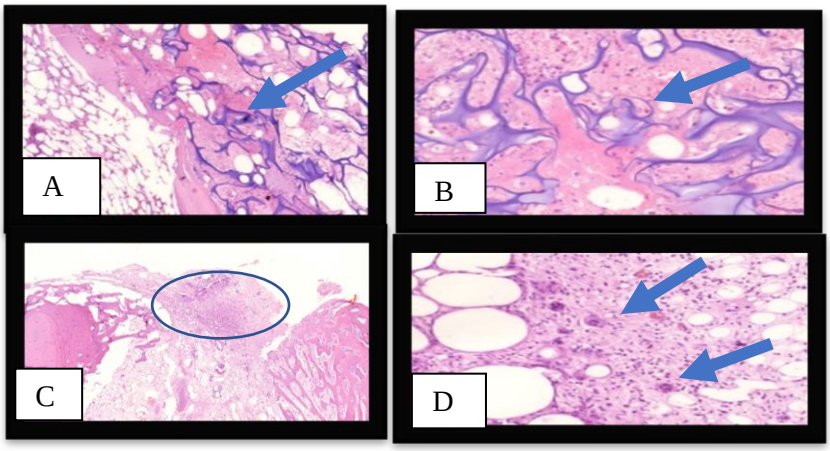


Fig7:-(A) At day 3 EA40 Mild early vascular granulation and moderate inflammation Marker pointing to HA (EA X40). (B) At day 14 of HA and PRP with moderate granulation with arow pointing to granulation tissue around implanted HA (EA X40). (C)At day 14 EA10 of PRP implanted site show mild granulation tissue (EA X10). (D)At day 21 EA PRP implanted site the arrows are pointing to the inflammation with multiple foreign body giant cells (EA X40).

At day 21, PRP, HA, and PRP & HA groups showed robust healing, while control group progress was limited. On day 28, control group had minimal healing, PRP group showed ongoing mild healing, and HA and PRP & HA groups displayed moderate healing(fig7). Biostatistical analysis highlighted significant differences between control, PRP, and HA groups at various days as shown in chart (1), indicating positive effects of PRP and HA on granulation tissue formation. No notable difference was seen between PRP and HA alone, and the combined effect was similar to PRP alone.

Inflammation Responses:

Histopathological analysis of inflammatory response in rabbit tibia bone defects revealed varying inflammation levels over different scarification days. At day 3, all groups displayed mild inflammation, reflecting initial responses to implanted materials. Control group consistently showed mild inflammation. PRP, HA, and PRP & HA groups exhibited similar mild to moderate inflammation, suggesting minimal alteration by PRP or HA alone.By day 7, control group maintained mild to moderate inflammation, as did PRP, HA, and PRP & HA groups. No significant change was seen in inflammation due to individual treatments.

At day 14, all groups demonstrated mild inflammation, indicating ongoing healing. No marked differences existed between groups.Day 21 showed similar inflammation patterns. PRP & HA's effect resembled PRP alone, suggesting no enhancement with combined treatment.By day 28, control group retained mild inflammation. PRP group had no inflammation, indicating potential healing with minimal inflammation. HA and PRP & HA groups showed reduced inflammation as shown in fig (8).

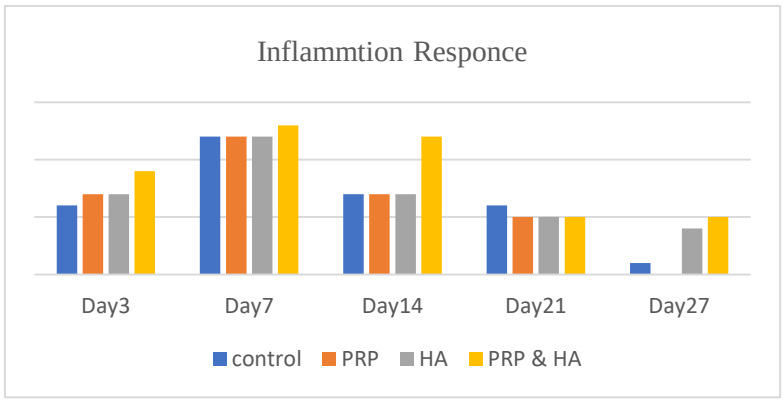
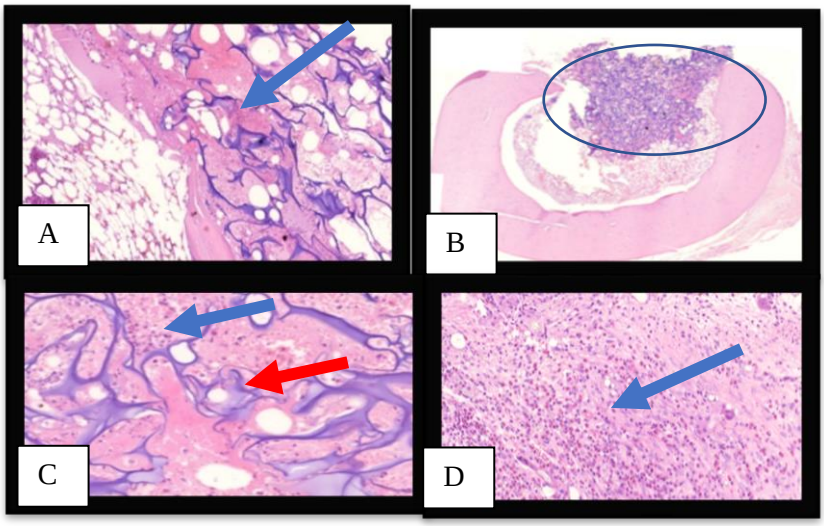


Chart 2:- Inflammations Response Analysis



Fig(8):- (A)AT day 3 HA and PRP show early mild vascular and inflammation surrounding HA on the location of marker (EA X40).(B) At day 7 showing the whole lesion the cycle shows the location of implant site (HA) with severe inflammation response (EA X10).(C) At day 14 the red arrow pointing to fibrin the blue arrow pointing to the inflammatory cells mainly neutrophils (EA X40).(D) At day 21 PRP and HA implanted site, arrow pointing abundant eosinophils in the inflammation (EA X40).

Biostatistical analysis indicated significant differences between control, PRP, and HA groups at various time points as shown in chart (2). PRP and HA reduced inflammation compared to control. No difference between PRP and HA, implying similar effects. Addition of HA to PRP didn't enhance inflammation reduction compared to PRP alone.

New bone formation: The criteria were used to evaluate the new bone formation showed that there was no significant difference between the different treatments at day 3 and day 7 in the bone formation. However, at day 14, there was a significant difference between the HA group and the HA mixed with PRP group, as well as between the HA and PRP group and the control group. At day 21 and day 28, there was a significant difference between all the treatments, with the HA and PRP group showing the high level of significancy of new bone formation as shown in chart (3) and F (9).

When analyzing the results of each hole that was implanted with different treatments, it can be seen that, there was a significant difference in new bone formation between the different treatments at day 14, day 21, and day 28. The HA and PRP group showed the highest level of new bone formation at all time points, followed by the HA group, the PRP group, and then the control group.

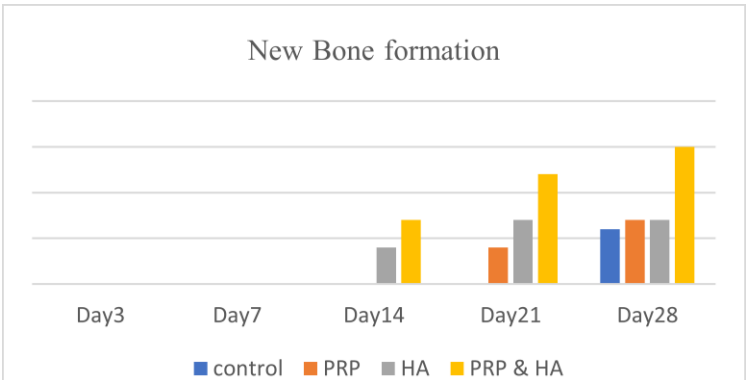


Chart3:- New Bone formation Analysis

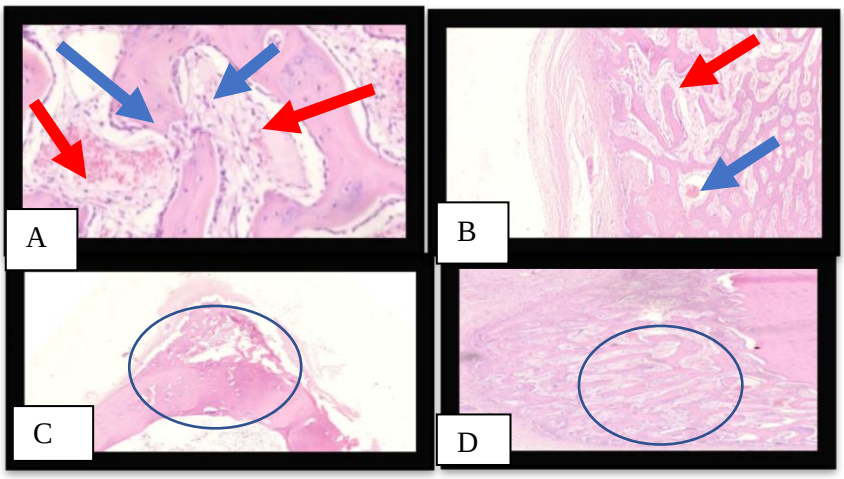


Fig. (9):-(A) AT day 14 from PRP The arrows are pointing to the osteoblasts line surface of bone trabeculae (EA X40), (B) At day 21 Bone formation. The blue arrows are pointing to the new bone formation. The red arrow is pointing to fibrous tissuethe.X4. From PRP(EA X40),(C)) At day 27 PRP and HA: Low power the cycle showing the new bone formation in the centre and the periphery(EA X10).(D)) At day 21 PRP and HA: A-New bone trabeculae(EA X40).

4-DISCUSSION

The utilization of Platelet-Rich Plasma (PRP) and Hyaluronic Acid (HA) in oral surgery has exhibited considerable potential for advancing bone regeneration and tissue healing. PRP, renowned for its concentrated platelet growth factors (PGFs), contributes significantly to the healing process by orchestrating migration, division, collagen

synthesis, and matrix formation in local mesenchymal and epithelial cells. This is due to its richness in autologous growth factors, including but not limited to vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1), insulin-like growth factor-2 (IGF-2), and transforming growth factor-β (TGF-β) (Everts et al., 2020; Fathi, 2012; Knox et al., 2006).

PRP demonstrates its effectiveness in soft tissue repair and alveolar bone regeneration, primarily within well-vascularized cancellous bone defects. The released growth factors from platelets in PRP initiate the recruitment of reparative cells and induce a spectrum of biological processes critical for tissue healing and alveolar bone regeneration (Yang et al., 2019). The secreted growth factors stimulate local mesenchymal and epithelial cells, driving migration, division, increased collagen synthesis, and matrix formation—culminating in the creation of fibrous connective tissue and scars (Sanchez et al., 2003). These growth factors work synergistically, interplaying with each other in damaged tissues, activating distinct intracellular signaling pathways, and enhancing the overall tissue repair process (Nikolidakis & Jansen, 2008).

Hyaluronic Acid (HA) complements this regenerative process by stabilizing matrices, inducing cell infiltration and migration, and regulating fibrin degradation. Its hydrophilic property, attracting and retaining water, establishes a moist environment essential for cell migration, tissue repair, and overall healing (Vasvani et al., 2020). Furthermore, HA's anti-inflammatory properties contribute to a conducive environment for bone repair by dampening the inflammatory phase (Schlundt et al., 2015).

However, PRP's impact on bone density may not be immediately significant during the initial healing weeks due to several factors. The absence of bone morphogenetic protein (BMP) in PRP could contribute to this non-significant difference, as BMP is established as a mediator in promoting bone formation during fracture healing (Sarkar et al., 2006). Moreover, PRP might necessitate more than two weeks to exert its effects on bone formation, a timeline supported by previous studies (Plachokova et al., 2006; Singh et al., 2017).

Similarly, HA's effects on bone density during the early phases of healing might be influenced by its need for time to facilitate healing. Factors like concentration, formulation, and application methods could also contribute to

non-significant differences in bone density during the initial healing stages. HA's properties, particularly in supporting cell migration, proliferation, and tissue remodeling, might become more pronounced at later stages of healing (Aslan et al., 2006; Park et al., 2016; Zhai et al., 2020).

The combination of PRP and HA showcases a synergistic effect on bone healing, primarily in later stages (14, 21, and 28 days). The cumulative and extended release of growth factors from PRP, coupled with HA's beneficial properties, contribute to increased bone density. Moreover, their anti-inflammatory properties and promotion of angiogenesis play pivotal roles in enhancing the bone healing process (Dhillon et al., 2017; chet et al., 2023; YU et al., 2018).

5-CONCLUSION

In conclusion, the use of PRP and HA in oral surgery presents a promising avenue for accelerating tissue regeneration and bone healing. Their individual properties, as well as their synergistic effects, contribute to granulation tissue formation, anti-inflammatory responses, and ultimately enhanced bone density. These findings suggest that PRP and HA can effectively improve the healing process in oral surgery, with their combined application demonstrating substantial potential for optimal outcomes.

6-REFERENCES

Abrahem SW, Al-Soudani KH (2012). Histopathological evaluation of aragonitic calcium carbonate as a bone graft substitute in rabbit's mandible (An experimental study). J Baghdad Coll Dent; 24: 58–61.

Anitua, E., Sánchez, M., & Orive, G. (2011). The importance of understanding what is platelet-rich growth factor (PRGF) and what is not. Journal of shoulder and elbow surgery, 20(1), e23–e24. https://doi.org/10.1016/j.jse.2010.07.005

Aslan M, Simsek G & Dayi E (2006). The effect of hyaluronic acid-supplemented bone graft in bone healing: experimental study in rabbits. Journal of biomaterials applications. 20(3), 209–220. https://doi.org/10.1177/0885328206051047

Casale, M., Moffa, A., Vella, P., Sabatino, L., Capuano, F., Salvinelli, B., Lopez, M. A., Carinci, F., & Salvinelli, F. (2016). Hyaluronic acid: Perspectives in dentistry. A systematic review. International journal of immunopathology and pharmacology, 29(4), 572–582. https://doi.org/10.1177/0394632016652906

Chen, C. H., Li, D. L., Chuang, A. D. C., Dash, B. S., & Chen, J. P. (2021). Tension stimulation of tenocytes in aligned hyaluronic acid/platelet-rich plasma-polycaprolactone core-sheath nanofiber membrane scaffold for tendon tissue engineering. International journal of molecular sciences, 22(20), 11215.

Council for International Organizations of Medical Sciences. (1985). International guiding principles for biomedical research involving animals. Altern Lab Anim., 12, ii.

Dhillon, M. S., Patel, S., & John, R. (2017). PRP in OA knee - update, current confusions and future options. SICOT-J, 3, 27. https://doi.org/10.1051/sicotj/2017004

Dimitriou, R., Jones, E., McGonagle, D., & Giannoudis, P. V. (2011). Bone regeneration: current concepts and future directions. BMC medicine, 9, 66. https://doi.org/10.1186/1741-7015-9-66

Elgendy EA, Shoukheba, MYM (2013). Histological and Histomorphometric Study of the Effect of Strontium Ranelate on the Healing of One-Wall Intrabony Periodontal Defects in Dogs. J Cytol Histol; 3(6): 1-6

Everts, P., Onishi, K., Jayaram, P., Lana, J. F., & Mautner, K. (2020). Platelet-Rich Plasma: New Performance Understandings and Therapeutic Considerations in 2020. International journal of molecular sciences, 21(20), 7794. https://doi.org/10.3390/ijms21207794

Fathi, W. K. (2012). The effect of hyaluronic acid and platelet-rich plasma on soft tissue wound healing: an experimental study on rabbits. Al-Rafidain Dental Journal, 12(1), 115-125.

Ghatak, S., Maytin, E. V., Mack, J. A., Hascall, V. C., Atanelishvili, I., Moreno Rodriguez, R., Markwald, R. R., & Misra, S. (2015). Roles of Proteoglycans and Glycosaminoglycans in Wound Healing and Fibrosis. International journal of cell biology, 2015, 834893. https://doi.org/10.1155/2015/834893

Gilat, R., Haunschild, E. D., Knapik, D. M., Evuarherhe, A., Jr, Parvaresh, K. C., & Cole, B. J. (2021). Hyaluronic acid and platelet-rich plasma for the management of knee osteoarthritis. International orthopaedics, 45(2), 345–354. https://doi.org/10.1007/s00264-020-04801-9

Gupta, R. C., Lall, R., Srivastava, A., & Sinha, A. (2019). Hyaluronic Acid: Molecular Mechanisms and Therapeutic Trajectory. Frontiers in veterinary science, 6, 192. https://doi.org/10.3389/fvets.2019.00192

Holmes MW, Bayliss MT, Muir H. Hyaluronic acid in human articular cartilage. Age-related changes in content and size. Biochem J. 1988

Mar 1;250(2):435-41. doi: 10.1042/bj2500435. PMID: 3355532; PMCID: PMC1148875.

Kim SY, Kim SG, Lim SC, Bae CS (2004). Effects on bone formation in ovariectomized rats after implantation of tooth ash and plaster of Paris mixture. J Oral Maxillofac Surg; 62: 852–5

Knox, R. L., Hunt, A. R., Collins, J. C., DeSmet, M., & Barnes, S. (2006). Platelet-rich plasma combined with skin substitute for chronic wound healing: a case report. The journal of extra-corporeal technology, 38(3), 260–264.

Litwiniuk, M., Krejner, A., Speyrer, M. S., Gauto, A. R., & Grzela, T. (2016). Hyaluronic acid in inflammation and tissue regeneration. Wounds, 28(3), 78-88.

Maeda, ST, Bramane CM, Taga R, Garcia RB, de Moraes IG, Bernadineli N (2007). Evaluation of surgical cavities filled with three types of calcium sulfate. J Appl Oral Sci; 15(5): 416-419.

Marques, L. F., Stessuk, T., Camargo, I. C. C., Sabeh Junior, N., Santos, L. D., & Ribeiro-Paes, J. T. (2015). Platelet-rich plasma (PRP): methodological aspects and clinical applications. Platelets, 26(2),

Maruyama, M., Rhee, C., Utsunomiya, T., Zhang, N., Ueno, M., Yao, Z., & Goodman, S. B. (2020). Modulation of the Inflammatory Response and Bone Healing. Frontiers in endocrinology, 11, 386. https://doi.org/10.3389/fendo.2020.00386

Marx R. E. (2001). Platelet-rich plasma (PRP): what is PRP and what is not PRP?. Implant dentistry, 10(4), 225–228. https://doi.org/10.1097/00008505-200110000-00002

Moshiri, A., & Oryan, A. (2013). Role of platelet rich plasma in soft and hard connective tissue healing: an evidence based review from basic to clinical application. Hard Tissue, 2(1), 6.

Nikolidakis D., Jansen J.A.. The biology of platelet-rich plasma and its application in oral surgery: literature review. Tissue Eng. Part B Rev. 2008;14:249–258

Park, H., Jin, Y., Shin, J., Yang, K., Lee, C., Yang, H., … & Cho, S. (2016). Catechol-functionalized Hyaluronic Acid Hydrogels Enhance Angiogenesis and Osteogenesis Of Human Adipose-derived Stem Cells In Critical Tissue Defects. Biomacromolecules, 6(17), 1939-1948. https://doi.org/10.1021/acs.biomac.5b01670

Pazzini JM, Nardi ABD, Huppes RR, Gering AP, Ferreira MG, Silveira CP & Santos R (2016). Method to obtain platelet-rich plasma from rabbits (Oryctolagus cuniculus). Pesquisa Veterinária Brasileira 36, 39-44

Plachokova, A., Dolder, J., Stoelinga, P., Jansen, J. (2006). The Bone Regenerative Effect Of Platelet-rich Plasma In Combination With An Osteoconductive Material In Rat Cranial Defects. Clin Oral Implants Res, 3(17), 305-311. https://doi.org/10.1111/j.1600-0501.2005.01208.x

Sanchez A.R., Sheridan P.J., Kupp L.I.. Is platelet-rich plasma the perfect enhancement factor? A current review. Int. J. Oral Maxillofac. Implants. 2003;18:93–103

Schlundt, C., Schell, H., Goodman, S., Vunjak-Novakovic, G., Duda, G., Schmidt-Bleek, K. (2015). Immune Modulation As a Therapeutic Strategy In Bone Regeneration. J EXP ORTOP, 1(2). https://doi.org/10.1186/s40634-014-0017-6

Singh, R., Rohilla, R., Gawande, J., & Kumar Sehgal, P. (2017). To evaluate the role of platelet-rich plasma in healing of acute diaphyseal fractures of the femur. Chinese journal of traumatology = Zhonghua chuang shang za zhi, 20(1), 39–44. https://doi.org/10.1016/j.cjtee.2016.03.007

Van Lieshout, E., & Den Hartog, D. (2021). Effect of platelet-rich plasma on fracture healing. Injury, 52 Suppl 2, S58–S66. https://doi.org/10.1016/j.injury.2020.12.005

Verma, R., Negi, G., Kandwal, A., Chandra, H., Gaur, D. S., & Harsh, M. (2019). Effect of autologous PRP on wound healing in dental regenerative surgeries and its correlation with PDGF levels. Asian journal of transfusion science, 13(1), 47–53. https://doi.org/10.4103/ajts.AJTS_25_17

Yang LC, Hu SW, Yan M, Yang JJ, Tsou SH, Lin YY. Antimicrobial activity of platelet-rich plasma and other plasma preparations against periodontal pathogens. J Periodontol 2015;86:310–318.

Yu, W., Xu, P., Huang, G., & Liu, L. (2018). Clinical therapy of hyaluronic acid combined with platelet-rich plasma for the treatment of knee osteoarthritis. Experimental and therapeutic medicine, 16(3), 2119-2125.

Zhai, P., Peng, X., Li, B., Liu, Y., Sun, H., & Li, X. (2020). The application of hyaluronic acid in bone regeneration. International journal of biological macromolecules, 151, 1224–1239. https://doi.org/10.1016/j.ijbiomac.2019.10.169

Zhai, P., Peng, X., Li, B., Liu, Y., Sun, H., & Li, X. (2020). The application of hyaluronic acid in bone regeneration. International journal of biological macromolecules, 151, 1224–1239. https://doi.org/10.1016/j.ijbiomac.2019.10.169

Zhang, X. L., Wang, Y. M., Chu, K., Wang, Z. H., Liu, Y. H., Jiang, L. H., ... & Yin, G. (2018). The application of PRP combined with TCP in repairing avascular necrosis of the femoral head after femoral neck fracture in rabbit. Eur Rev Vet Med Pharm Sci, 22, 903-90