EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
VOL. 68, 1423:1433, APRIL, 2022
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
ORAL SURGERY
Submit Date : 26-02-2022 • Accept Date : 31-03-2022 • Available online: 30-03-2022 • DOI : 10.21608/edj.2022.121264.1989

THE EFFECT OF BIOACTIVE HYDROGEL VERSUS ADVANCED-PRF ON BONE REGENERATION FOLLOWING IMPACTED MANDIBULAR THIRD MOLAR SURGERY. CLINICAL AND CBCT ANALYSIS

Shereen W. Arafat*, Ahmed M Hossam Eldin**, Nourhan A Hussien***, Rania F. Abdulmaguid**** *and* Mohamed A. Elbaz*****

ABSTRACT

Objectives: The current study aim was to evaluate the effect of Bioactive Hydrogel versus Advanced-PRF on bone regeneration following impacted mandibular third molar surgery.

Material & Methods: sixty patients who had been scheduled for surgical removal of mesio-angular or horizontal impacted mandibular 3rd molar teeth were randomly divided into three groups. Group A involved 20 patients received A-PRF-Xenograft at the surgical site. Group B involves 20 patients received Xenograft-Hydrogel at the surgical site. While group C involved 20 patients left to heal without graft as control. Clinical and radiographic evaluation were performed immediate and at 6 months postoperatively. Data was collected and analysed statistically.

Results: At 6-month postoperative evaluation, the current study found that A-PRF-Xenograft group showed significant shorter mean pain duration (5.1±1.4 days) compared to Hydrogel-Xenograft group (5.3±1.5 days) and control group (5.8±1.4 days). A-PRF-Xenograft group showed the lowest mean values of Periodontal Pocket Depth (PPD) (3.7±0.6) followed by Hydrogel-Xenograft group (5±0.8) and followed by control group (6.6±0.6). A-PRF-Xenograft group showed the highest mean values of bone density (605.3±85.5) followed by Hydrogel-Xenograft group (522.4±83.5) followed by control group (286.4±44.7)

Conclusion: According to the results of the present study, there was significant improvement regarding pain duration, mouth opening limitation, periodontal pocket depth and bone density at the surgical site where A-PRF-Xenograft was applied. Hydrogel-Xenograft Group was more superior to the control group regarding pain duration, PPD, and bone density.

* Associate Professor of Oral & Maxillofacial Surgery, October University for Modern Sciences & Arts, Egypt.
** Associate Professor of Oral Radiology, October University for Modern Sciences & Arts, Egypt.
*** Teaching Assisstant of Oral & Maxillofacial Surgery, October University for Modern Sciences & Arts, Egypt.
**** Associate Professor of Oral Medicine & Periodontology, October University for Modern Sciences & Arts, Egypt.
***** Associate Professor of Oral & Maxillofacial Surgery, October University for Modern Sciences & Arts, Egypt.

INTRODUCTION

Introduction:

Surgical removal of impacted third molar is a routine practice for oral and maxillofacial surgeons. Mesio-angular and horizontal third molars are considered among the most unfavourable positions of impacted mandibular third molars regarding postoperative complications, pain and sensitivity as usually associated with osseous defects distal to the second molar and/or the development of periodontal defects. [1-4] Moreover, the surgical removal of impacted third molar may lead to difficulty in maintaining proper oral hygiene, and subsequently gradual bone loss in this area. [5] Therefore, it is an essential concern to minimize the postoperative complications and enhance bone healing at the third molar socket. It was reported that after third molar surgical removal, bone healing may be delayed up to 4 years although the most significant change takes place after 3 months. [6,7]

Several research have been conducted on grafting the third molar socket with different materials to improve bone regeneration, and reported that grafting is more effective than natural healing regarding the postoperative complications. [8,9] These materials could be autogenous bone, allogeneic bone, bone graft substitutes or a combination of these materials. [10]

The allografts are widely used due to their ease of use and unlimited availability. Allogeneic bone graft must undergo deproteinization and decellularization to ensure immune-compatibility. [11] The lack of osteoprogenitor cells, and pro-osteogenic proteins in the allografts causes slow bone regeneration and delayed graft incorporation. Accordingly, if the rate of new bone formation could be accelerated around allografts, better results could be obtained. [12]

In 2000, Choukroun [13] introduced PRF by using a firm consistency of platelet-rich concentrate which is known as the second-generation platelet concentration. Then the PRF variants were introduced which are advanced PRF (A-PRF) by Choukroun[14], and injectable PRF (I-PRF) by Muorao. [15]

A-PRF is rich in retained leukocytes owing to its slow centrifugation and a more porous fibrin matrix which in turn causes more release of its contents. The higher porosity causes more penetration of blood vessel during angiogenesis. Moreover, the literature reported the major role of angiogenesis during bone regeneration. The newly formed blood vessels are excellent source of nutrients, growth factors, stem cells and progenitors. [16,17] Furthermore, this form of PRF is malleable, adaptable and can be cut into smaller pieces for bone grafting or used as a membrane. Also, the fibrin matrix acts as a scaffold for the leukocytes and platelets and their release products. [18] It was reported that A-PRF was shown to release significantly higher quantities of growth factors as compared to traditional PRF in vitro. [19]

One of the emerging eras in regenerative medicine is hydrogels. They are composed of three-dimensional hydrophilic polymer chains, which in turn have superior mechanical strength and can provide nutrient environments for endogenous cell growth. [20] Moreover, hydrogels are absorbable and can integrate with surrounding tissues. [21]

The use of hydrogels for preparing bone grafts have several benefits for bone regeneration owing to their swollen network structures that can contain biologically active agents and excellent biocompatibility. Moreover, the three-dimensional hydrophilic characteristics of hydrogels causes higher mechanical strength and nutritional environments for the growth of endogenous cell. Furthermore, hydrogel is viscoelastic with soft texture and decreases the inflammatory responses. [22-24]

One of the essential difficulties in grafting irregularly shaped or large defects is to maintain particle-type bone graft materials stably, and prognoses are relatively poor. Consequently, the researches now are conducted to the use of A-PRF

added to xenograft to form sticky bone or hydrogel containing xenograft in form of sticky bone. [25]

Therefore, the current study evaluates the effect of Bioactive Hydrogel versus Advanced-PRF on bone regeneration following impacted mandibular third molar surgery.

Cone beam computed tomography (CBCT) has become a commonly accepted diagnostic tool, as it offers extremely accurate 3D diagnostics allowing for small Fields of View (FOV), good image quality, and low radiation doses. [26]

The introduction of 3-dimensional (3D) cone beam computed tomography (CBCT) imaging to enhance preoperative treatment planning has proved valuable. CBCT has a reasonably low radiation dose and a high spatial resolution. Reports regarding the accuracy and reliability of linear measurements of the CBCT exist. Study outcomes demonstrate the reliability and validity of CBCT to assess postoperative outcomes to detect dimensional and qualitative changes where bone regeneration has been attempted in normal and bone-compromised individuals. [27]

MATERIALS & METHODS

A total of 60 patients (28 females and 32 males) aged 19 – 35 years who had been scheduled for surgical removal of their impacted mandibular third molars at the Dental Clinics of October University of Modern Sciences and Art were selected. All patients were informed and signed a written consents for sharing in this research according to the Committee of Ethics of Faculty of Dentistry, October University of Modern Sciences and Art.

Each patient was assessed clinically and radiographically (preoperative panorama). The main selection criteria were absence of systemic disease, non-smokers, good oral hygiene, and the presence of mesioangular or horizontal impacted mandibular third molars. While patients with malignancies, maxillofacial syndromes, and patients having root caries of a second molar and/or pericoronitis of the third molar were excluded from the study.

All selected patients received 12% chlorhexidine as mouthwash for 1 min, and an extraoral antisepsis with 1% topical povidine. They were randomly allocated into either groups A or B or C. Twenty patients of group A (study group A) underwent surgical removal of impacted mandibular third molar followed by application of A-PRF mixed with xenograft in the surgical site. Twenty patients of group B (study group B) underwent surgical removal of impacted mandibular third molar followed by application of xenograft containing hydrogel in the surgical site. Twenty patients of group C (control group) underwent surgical removal of impacted mandibular third molar only. Surgical extractions were carried out by the same surgeon, the radiographic and periodontal measurements were performed by the same radiologist and periodontist.

Surgical procedure:

All patients were prepared for surgery by 0.12% chlorexidine mouthwash for 1 min. Inferior alveolar, lingual, and long buccal nerves were anesthetized by 2% mepivacaine/1: 100 000 epinephrine. A mucoperiosteal envelope flap with a releasing incision anterior to the second molar was performed according to Rosa et al. [28] After elevation of the mucoperiosteal flaps, bone removal and tooth sectioning were accomplished using low-speed surgical bur with copious irrigation to facilitate removal. Then surgical site debridement was performed to remove any remaining dental follicle at the 3rd molar socket, and root planning of the exposed distal root surfaces of the second molars was achieved by hand instruments.

A-PRF-Xenograft group (test group A):

Venous blood was withdrawn in 10 mL glass tubes without anticoagulants. Samples were immediately centrifuged at 200 g for 8 min. [29] The red blood cell fraction was separated with scissors and discarded, while the remaining A-PRF clot was placed on a

dry gauze to remove excess serum and incubated for 10 min at room temperature. A-PRF clot was cut into 1-2 mm PRF fragments and mixed with 0.25 g of DBBM (Bio-Oss®, Geistlich, Wolhusen, Switzerland). Gentle stirring was performed for 15 seconds for shaping into a sticky bone block. [30] Finally, the formed sticky bone was placed into the extraction socket. Fig. 1

Hydrogel-Xenograft group (test group B)

The bone graft particles S1-XB (S1-XB®, Medpark, Busan, Korea) were hydrated with saline in a tray, and stirred for at least 30 seconds to form a lumpy shape. The formed sticky bone was applied into the extraction socket. Fig. 2

Control group (group C)

The extraction sockets left to heal without bone graft. Finally, the mucoperiosteal flaps were repositioned, and sutured with 3-0 resorbable Vicryl chromic suture (Johnson & Johnson, New Brunswick, NJ). All patients received antibiotic (1g/12 h augmentin; GlaxoSmithKline S.A.E, Cairo, Egypt), NSAIDs (400 mg, twice daily ibuprofen; Kahira Pharmaceuticals and Chemical Industries Company, Cairo, Egypt), and 0.12% chlorhexidine gluconate oral rinse twice daily. Patients were examined 1 week postoperatively by the surgeon to ensure proper surgical healing.

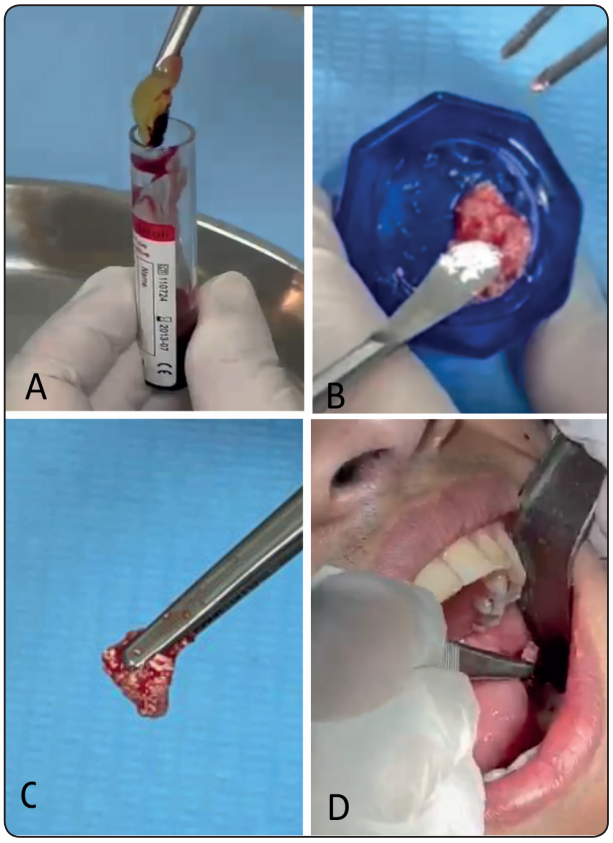

Fig. (1) Case no. 4 in study group A (A-PRF-Xenograft), A: A-PRF after centrifugation, B: the A-PRF after cutting into pieces and mixing with the Xenograft, C: the formed sticky bone graft, D: the A-PRF-Xenograft placement in the empty socked after impacted 3rd molar surgical removal

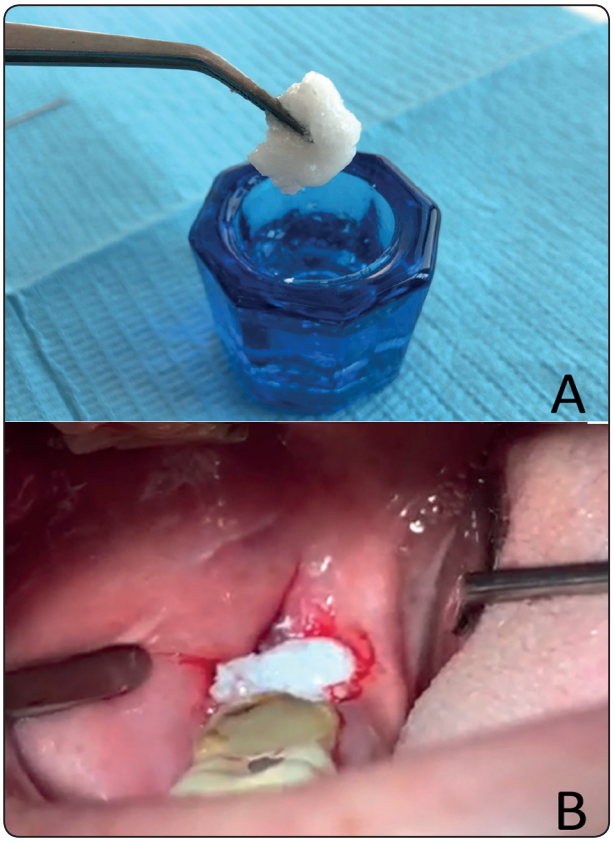

Fig. (2): Case no. 7 in study group B (Hydrogel-Xenograft), A: mixing the graft with the recommended amount of saline to form sticky bone, B: the sticky bone placement in the empty socked after impacted 3rd molar surgical removal

Clinical evaluation:

Post operative pain score and duration as well as the mouth opening limitation were recorded by each patient through a questionnaire at the one week follow up visit.

The probing pocket depth (PPD) was measured and recorded immediate and at 6 months postoperatively. The measurements were taken using a 'Williams's graduated probe (0.5 mm of tip diameter; PQWBR; Hu-Friedy do Brasil, Rio de Janeiro, Brazil). The probe was placed parallel to the mandibular second molar long axis at the gingival sulcus till resistance was felt. The measurements were taken at the mid-distal, distobuccal, and distolingual site from the cementoenamel junction then the average PPD was calculated to the nearest millimetre.

Radiographic evaluation

Cone Beam Computed Tomography (CBCT) was performed immediate and at 6 months postoperatively for each patient. The raw data obtained from the CBCT scanning were imported to the On-Demand 3D software for secondary reconstruction (OnDemand3D, version 1.0.9; Cybermed, Seoul, South Korea). To optimize visualization, images were magnified 3x to delineate the bone structure at high resolution. The radiologist performed image analysis in a blind and independent fashion. Analysis was performed twice, at two different sessions with a two-week interval in between the sessions.

To determine the marginal bone defect level, length at the distal side of second lower molars was measured. The distance from the cementoenamel junction to the bottom of the defect (alveolar bone crest) was determined at distobuccal, mid-distal, and distolingual of the lower second molar immediately after surgery and at 6 months postoperatively. For bone density, pixel intensity values were measured in squares of 30 × 30 pixels in six different points at the distal of lower second molar, and the average of the six points was calculated. This was performed immediately after surgery and at 6 months postoperatively. Fig. 3

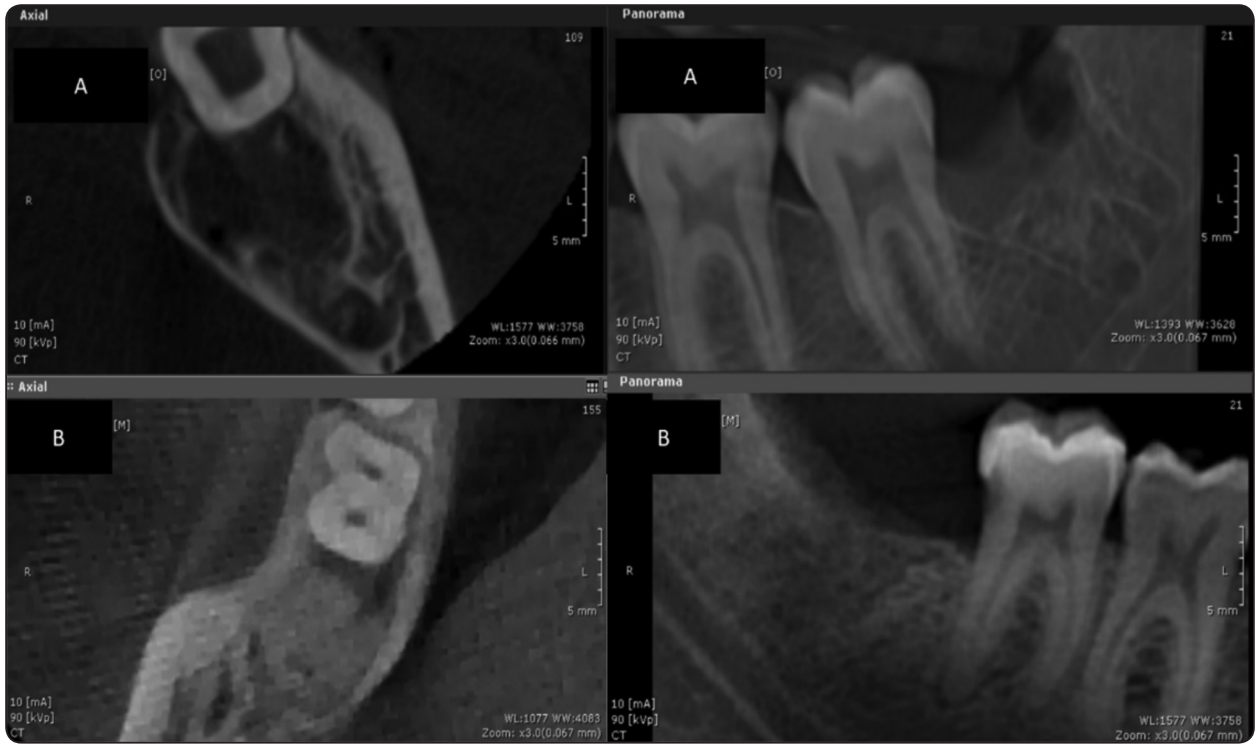

Fig. (3): A: Axial & Panoramic crop (Immediate post operative), B: Axial & Panoramic crop (6 month postoperatively)

To ensure the reliability and reproducibility of the results, all measures were taken twice by the same radiologist, and periodontist, and their mean values were calculated.

Statistical Analysis

Data presented as mean and standard deviation (SD). Data explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. PDD, pain score and duration showed non-normal distribution, Kruskal Wallis test used to compare between tested groups followed by Mann Whitney U test for pairwise comparison with Dunn Bonferroni correction. Wilcoxon singed rank test used to compare between follow-up periods. Bone defect length and density showed normal distribution so paired t-test used to compare between follow-up period and one way ANOVA used to compare between tested groups followed by pairwise comparison with Tukey HSD test. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

RESULTS

In the current study, the clinical and radiographic data of 60 patients including 20 patients who received A-PRF-Xenograft and 20 patients who received Hydrogel-Xenograft and 20 patients who didn't receive bone graft after surgical removal of impacted mandibular third molar were analysed and presented.

The age of the study patients ranged from 19 to 35 years. Clinical evaluation of the postoperative healing revealed an excellent soft tissue response to all treatment modalities without any complications or adverse reactions.

Analyses of post-operative pain score and duration

There was insignificant difference between the study groups regarding pain score at p=0.371. While A-PRF-Xenograft group showed significant shorter mean pain duration (5.1±1.4 days) compared to Hydrogel-Xenograft group (5.3±1.5 days) and control group (5.8±1.4 days). Fig. 4

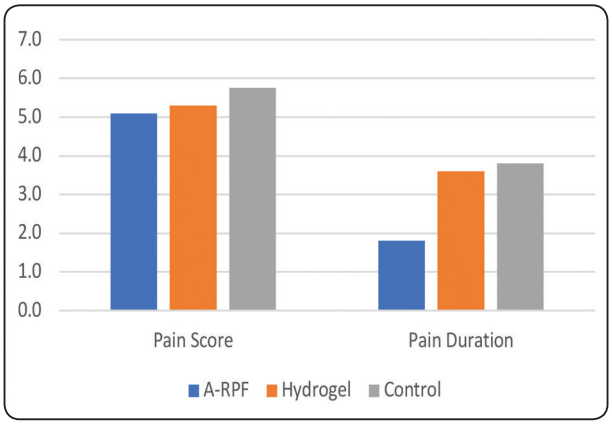

Fig. (4): Postoperative pain score and duration for the study groups

Analysis of postoperative mouth opening limitation

A-PRF-Xenograft group showed only 15% (n=3) limited mouth opening during the postoperative 1st week. While both Hydrogel-Xenograft and control groups showed 35% (n=13) with insignificant difference between the study groups (p=0.269). Fig. 5

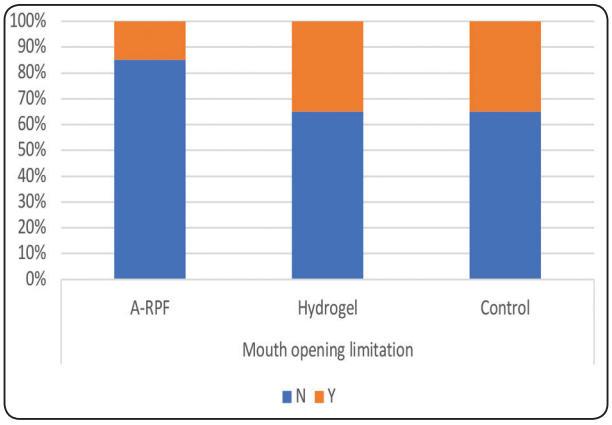

Fig. (5): Postoperative mouth opening limitation for the study groups.

Analysis of Periodontal Pocket Depth

All tested groups showed significantly lower PPD values at 6 months evaluation. At immediate evaluation, Insignificant difference resulted between

tested groups at p=0.974. While after 6 months evaluation, A-PRF-Xenograft group showed the lowest mean values of PPD (3.7±0.6) followed by Hydrogel-Xenograft group (5±0.8) and followed by control group (6.6±0.6). Fig. 6

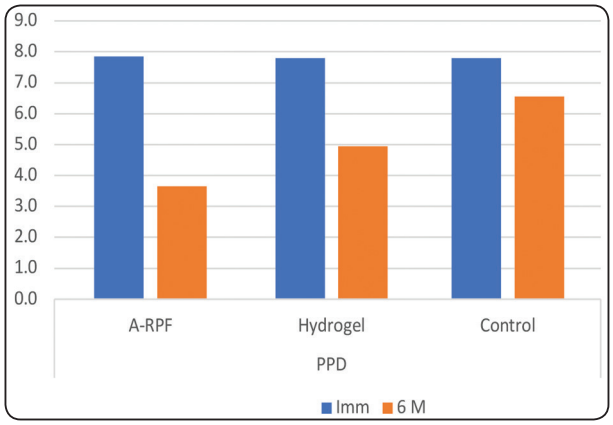

Fig. (6): The Periodontal Pocket Depth (PPD) of the study groups immediate and at 6 months postoperatively.

Analysis of defect length:

At immediate evaluation and after 6 months, Insignificant difference resulted between the study groups at p=0.157. All tested groups showed significantly lower mean bone defect length (mm) values at 6 months evaluation. Table 1

TABLE (1): Mean and SD for bone defect length (mm) for the study groups for Follow-up periods

		Imm		6 M		p-value
		Mean	SD	Mean	SD	
bone defect length(mm)	A-RPF	5.7	0.9	3.1	0.5	0.005*
	Hydrogel	5.5	1.2	3.2	1.0	<0.001*
	Control	5.1	0.9	3.4	0.8	<0.001*
p-value		0.157 NS		0.484 NS		

Different letters within each column indicates significant difference

NS= Non-significant, *= significant

Analysis of bone density:

At immediate evaluation, insignificant difference resulted between the study groups at p=0.185. While at 6 months evaluation, A-PRF-Xenograft group showed the highest mean values of bone density (605.3±85.5) followed by Hydrogel-Xenograft group (522.4±83.5) followed by control group (286.4±44.7). Fig. 7

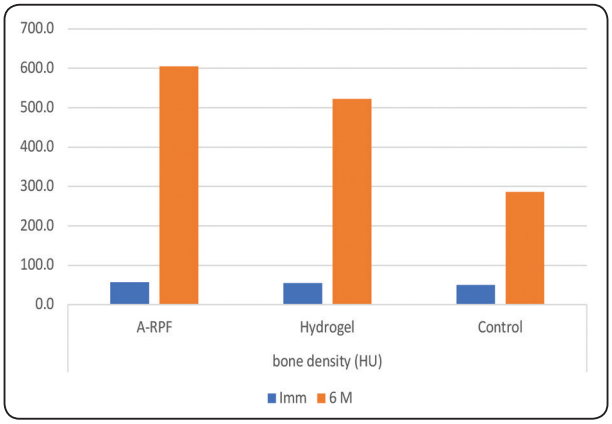

Fig. (7): Bone density values (HU) immediate and at 6 months postoperatively of the study groups.

DISCUSSION

After surgical removal of an impacted mandibular third molar, there is always debate on whether or not a reconstructive treatment is necessary. Various reconstructive procedures have been studied extensively for their therapeutic effects to enhance bone healing and minimize post-operative complications.

Several clinical and histologic studies have recommended the use of anorganic bovine bone graft for bone regeneration. [31-34] Xenograft provides a framework onto which bone-forming cells, and blood vessels can migrate to produce healthy new bone. Moreover, Innovation in the PRF technique of preparation known as Advanced Platelet rich fibrin (A-PRF) was chosen in the current study to be additive regenerative material to xenograft based on the superiority reported as highest growth factors release. [35]

The postoperative complications and discomfort are the major worries for people considering

surgical removal of impacted third molars in terms of pain, swelling, and trismus. Therefore, post-operative complications reduction is a crucial indicator for the surgical extraction's overall success.

In the current study, A-PRF-Xenograft group showed significant shorter mean pain duration as compared to Hydrogel-Xenograft group and control group. Moreover, A-PRF-Xenograft group showed only 15% limited mouth opening during the postoperative 1st week as mouth opening less than 35 mm was considered limitation. While both Hydrogel-Xenograft and control groups showed 35%. These findings signify the role of A-PRF in reducing post-operative pain duration, and mouth opening limitation. Same results were reported by Simon et al. [36] using PRP gel in third molar extraction sockets. Moreover, Kumar et al. [37] reported in their study on impacted mandibular 3rd molar socket that the use of PRF has affected the mouth opening limitation positively. Furthermore, it was reported by Del Fabbro et al. [38] that autologous platelet concentrates decrease postoperative pain and discomfort when applied in extraction sockets. In another study by Arenaz-Búa et al.[39], it was found that the groups with less trismus were the groups where Platelet concentrates applied.

In the current study, the improvement in pain score was Insignificant between the study groups although A-PRF-Xenograft showed less scores followed by Hydrogel-Xenograft group followed by control group. This finding contradicts with that of Ogundipe et al. [40] who reported that PRP group had minimized pain, swelling, and trismus, but this improvement was statistically important only for pain. This could be attributed to the varied platelet concentrate used with the different technique of preparation for each.

The surgical removal of mesioangular/horizontal mandibular impacted third molars are usually associated with periodontal defect at the distal surface of the second molar which is reported as disruption of periodontal ligaments and pocket depth with attachment loss. [41,42]

In the current study, A-PRF-Xenograft group showed the lowest mean values of PPD followed by Hydrogel-Xenograft group and followed by control group. This indicates better periodontal healing in the study group A. This finding is in accordance with other studies which reported the role of platelet concentrates in accelerating bone and periodontium regeneration. [43,44] Moreover PRF placement after extraction of mandibular third molars was reported to enhance healing and minimize the potential postoperative complications. [45,46] Furthermore, Kan et al. [47] reported in his study that healing of the periodontium on the distal surface of mandibular 2nd molar took 6 months after surgical removal of the impacted 3rd molar.

Regarding the study group B, the current study reported that it was superior to control group regarding the PPD. This finding could be explained based on the previous studies which reported that Hydrogels plays an essential role in bone regeneration with its mechanical strength, nutritional conditions that are conducive to endogenous cell proliferation and also it reduces the inflammatory reactions. [48-50]

In the current study, all the study groups showed significantly lower mean bone defect length after 6 months of healing with insignificant difference between the groups. While A-PRF-Xenograft group showed the highest mean values of bone density followed by Hydrogel-Xenograft group followed by control group. This radiographic analysis corresponds to the clinical analysis of our study and signifies that A-PRF-Xenograft was superior to the other groups and the Hydrogel-Xenograft group was superior to the control group.

Our finding could be explained by the previous studies which reported that PRF has slow polymerization during its preparation which generates a fibrin network near to the natural one. This in turn enhances cell migration and proliferation. Moreover, PRF acts as a reservoir of platelets, leukocytes, cytokines and immune cells, and slowly releases the cytokines; TGF,

PDGF, VEGF, and EGF which are essential for angiogenesis and tissue healing. [51,52]

These findings contradict with the findings of Gurbuzer et al. [53] who reported that PRF has the potential characteristics of an autologous fibrin matrix, but may not improve bone healing. This difference could be attributed to the variation of PRF preparation technique mentioned in their study.

It has been clear in the current study that Hydrogel-Xenograft group was superior to the control group. This finding was supported by the findings of other studies which reported that hydrogel promotes spreading, proliferation, and differentiation of mesenchymal stem cells. [54] Moreover, it was reported that the use of hydrogels for preparing bone grafts have several benefits for bone regeneration as their swollen network structures and three-dimensional hydrophilic characteristics provide mechanical strength and nutritional environments for endogenous cell growth. [55]

Finally, this study showed that application of bone graft to the third molar extraction socket can provide superior results in terms of decreasing the post-extraction pain duration, and mouth opening limitation, and increasing bone density rather than leaving the extraction socket without grafting.

CONCLUSION

According to the findings of the present study, we can conclude that A-PRF-Xenograft was superior in reducing postoperative complications in terms of pain duration, and mouth opening limitation, and also in decreasing periodontal pocket depth and increasing bone density when applied at the surgical site. On the other hand, A-PRF-Xenograft showed no clear benefit regarding pain score, and defect length on the distal surface of second molar tooth. Moreover, Hydrogel-Xenograft Group was superior to the control group regarding pain duration, PPD, and bone density. However, long-term, multicenter, randomized, controlled clinical trials are required.

REFERENCES

1. Oxford, G.E., Quintero, G., Stuller, C.B., and Gher, M.E.: Treatment of 3rd molar-induced periodontal defects with guided tissue regeneration. J Clin Periodontol., 24:464–469, 1997.
2. Ash, M., Costich, E. and Hayward, J.: A study of periodontal hazards of 3rd molars. J Periodontol., 33:204–209, 1962.
3. Szmyd, L. andHester, W.R., Crevicular depth of the second molar in impacted third molar surgery. J. Oral Surg. Anesth. Hosp. Dent. Serv., 21:185–189., 1963.
4. Grondahl, H.G. and Lekholm, U., Influence of mandibular third molars on related supporting tissues. Int. J. Oral Surg., 2:137–142, 1973.
5. Motamedi, M.H.: A technique to manage gingival complications of third molar surgery. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod., 90:140, 2000.
6. Chen, Y.W., Lee, C.T., Hum, L. and Chuang, S.K.: Effect of flap design on periodontal healing after impacted third molar extraction: A systematic review and meta-analysis. Int. J. Oral Maxillofac. Surg., 46:363, 2017.
7. Kugelberg, C.F.: Periodontal healing two and four years after impacted lower third molar surgery. A comparative retrospective study. Int. J. Oral Maxillofac. Surg. 19:341, 1990.
8. Araujo, M.G. and Lindhe, J.: Ridge preservation with the use of Bio-Oss (R) collagen: a 6-month study in the dog. Clin. Oral Implants Res. 20:433-40, 2009.
9. Park, J-Y, Koo, K-T and Kim, T-I.: Socket preservation using deproteinized horse-derived bone mineral. J. Perio. Implant Sci. 40:227-31, 2010.
10. Khalid, S. H., Hesham, F. M., and Adel, S. A.: Does Grafting of Third Molar Extraction Sockets Enhance Periodontal Measures in 30- to 35-Year-Old Patients? J. Oral Maxillofac. Surg. 70:757-764, 2012.
11. Tuli, S.M. and Singh, A.D.: The osteoninductive property of decalcified bone matrix. An experimental study. J. Bone Joint Surg. Br. 60(1):116–23, 1978.
12. Tilkeridis, K., Touzopoulos, P., Ververidis, A., Christodoulou, S., Kazakos, K. and Drosos, G.I.: Use of demineralized bone matrix in spinal fusion. World J. Orthop. 5(1):30–7, 2014.
13. Choukroun, J., Adda, F. and Schoeffer, C.: PRF: an opportunity in perio-implantology. Implant odontie, 42:55–62, 2000.

14. Choukroun, J. and Ghanaati, S.: Reduction of relative centrifugation force within injectable platelet-rich-fibrin (PRF) concentrates advances patients' own inflammatory cells, platelets and growth factors: the first introduction to the low-speed centrifugation concept. Eur. J. Trauma Emerg. Surg., 44(1):87–95, 2018.
15. Moura˜o, C.F., Valiense, H., and Melo, E.R.: Obtention of injectable platelets rich-fibrin (i-PRF) and its polymerization with bone graft: technical note. Rev. Col. Bras. Cir.,42:421–3, 2015.
16. Miron, R.J., Fujioka-Kobayashi, M., Bishara, M., Zhang, Y., Hernandez, M. and Choukroun. J.: Platelet-rich fibrin and soft tissue wound healing: a systematic review. Tissue Eng. B. Rev., 23:83-99, 2017.
17. Kurobane, T., Shiwaku, Y., Anada, T., Hamai, R., Tsuchiya, K. and Baba, K.: Angiogenesis involvement by octacalcium phosphate-gelatin composite-driven bone regeneration in rat calvaria critical-sized defect. Acta. Biomater, 88: 514-26, 2019.
18. Yijiao, F., Karla, P., and Harry, D.: Clinical Uses of Platelet-Rich Fibrin in Oral and Maxillofacial Surgery. Dent. Clin. N. Am, 64: 291–303, 2020.
19. Kobayashi, E., Flückiger, L., Fujioka-Kobayashi, M., Sawada, K., Sculean, A. and Schaller, B.: Comparative release of growth factors from PRP, PRF, and advanced-PRF. Clin. Oral Invest., 20:2353-2360, 2016.
20. Wu, G., Feng, C., Quan, J., Wang, Z., Wei, W., Zang, S., Kang, S., Hui, G., Chen, X., and Wang, Q.: In situ controlled release of stromal cell-derived factor-1alpha and antimiR-138 for on-demand cranial bone regeneration. Carbohydr. Polym., 182: 215-224, 2018.
21. Silva, R., Fabry, B. and Boccaccini, A.R.: Fibrous protein-based hydrogels for cell encapsulation. Biomaterials, 35: 6727-6738, 2014.
22. Gibbs, D.M., Black, C.R., Dawson, J.I., Oreffo, R.O.: A review of hydrogel use in fracture healing and bone regeneration. J. Tissue Eng. Regen. Med., 10: 187–198, 2016.
23. Bai, X., Gao, M., Syed, S., Zhuang, J., Xu, X. and Zhang, X.-Q.: Bioactive hydrogels for bone regeneration. Bioact. Mater., 3: 401–417, 2018.
24. Buwalda, S.J., Vermonden, T. and Hennink, W.E.: Hydrogels for therapeutic delivery: Current developments and future directions. Biomacromolecules, 18, 316–330, 2017.
25. Buser, D., Dula, K., Belser, U., Hirt, H.-P. and Berthold, H.: Localized ridge augmentation using guided bone regeneration. I. Surgical procedure in the maxilla. Int. J. Periodontics Restor. Dent., 13: 29–45, 1993.
26. Chappuis, V., Engel, O., Reyes, M., Shahim, K., Nolte, L.-P., and Buser, D.: Ridge Alterations Post-extraction in the Esthetic Zone: A 3D Analysis with CBCT. JDR Clinical Research Supplement., 92(2): 195-201, 2013.
27. Tadinada, A., Ortiz, D., Taxel, P., Shafer, D., Rengasamy, K., Pendrys, D., and Freilich, M.: CBCT evaluation of buccal bone regeneration in postmenopausal women with and without osteopenia or osteoporosis undergoing dental implant therapy. J. Prosth. Dent., 114(4):498-505, 2015.
28. Rosa, A.L., Carneiro, M.G., Lavrador, M.A. and Novaes, A.B.: Influence of flap design on periodontal healing of second molars after extraction of impacted mandibular third molars. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod., 93:404–407, 2002.
29. To, M., Su, C.Y., Hidaka, K., Okudera, T. and Matsuo, M.: Effect of advanced platelet-rich fibrin on accelerating alveolar bone formation in dogs: a histological and immunofluorescence evaluation. Anat. Sci. Int., 94:238-44, 2019.
30. Fenga, M., Wanga, Y., Wei, Y., Zhanga, X., Xiaoa , L., Gonga, Z., Fujioka-Kobayashi, M., Sculeanc, A., Mironc, R.J., Froumd, S., and Zhanga, Y.: Preparation, characterization and biological properties of a novel bone block composed of platelet rich fibrin and a deproteinized bovine bone mineral. Fundamental Research, 2021. https://doi.org/10.1016/j.fmre.2021.08.003.
31. Older, L.B.: The use of heterogenous bovine bone implants in the treatment of periodontal pockets. An experimental study in humans. J Periodontol., 38:539-45 1967.
32. Arrocha, R., Wittwer, J.W., and Gargiulo, A.W.: Tissue response to heterogeneous bone implantation in dogs. J Periodontol., 39:40-48, 1968.
33. Nielsen, I.M., Ellegaard, B., and Karring, T.: Kiel bone in healing interradicular lesions in monkeys. J Periodont Res., 15:328-33, 1980.
34. Nielsen, I.M., Ellegaard, B., and Karring, T.: Kiel bone in new attachment attempts in humans. J Periodontol., 52:723-29, 1981.
35. Yewale, M., Bhat, S., Kamath, A., Tamrakar, A., Patil, V. and Algal, A.S.:Advanced platelet-rich fibrin plus and osseous bone graft for socket preservation and ridge augmentation–A randomized control clinical trial. J Oral Biology and Craniofac Res., 11(2):225-233, 2021.
36. Simon, B.I., Zatcoff, A.L., Kong, J.J., and O'Connell, S.M.: Clinical and histological comparison of extraction socket healing following the use of autologous platelet-rich fibrin matrix (PRFM) to ridge preservation procedures employing demineralized freeze dried bone allograft material and membrane. Open Dent J., 3:92–9, 2009.

37. Kumar, N., Prasad, K., Ramanujam, L., Ranganath, K., Dexith, J. and Chauhan, A.: Evaluation of treatment outcome after impacted mandibular third molar surgery with the use of autologous platelet-rich fibrin: a randomized controlled clinical study. J Oral Maxillofac Surg., 73(6):1042-1049, 2015.

38. Del Fabbro, M., Brotolin, M., and Taschieri, S.: Is autologous platelet concentrate beneficial for post-extraction socket healing? A systematic review. Int J Oral Maxillofac Surg., 40:891-99, 2011.

39. Arenaz, B.J., Luaces, R.R., Sironvalle, S.S., Otero, R.A., Charro, H.E., and Patiño, S.B.: A comparative study of platelet-rich plasma, hydroxyapatite, demineralized bone matrix and autologous bone to promote bone regeneration after mandibular impacted third molar extraction. Med Oral Patol Oral Cir Bucal., 15:483–9, 2010.

40. Ogundipe, O.K., Ugboko, V.I., Owotade, F.J.: Can autologous platelet-rich plasma gel enhance healing after surgical extrac tion of mandibular third molars? J Oral Maxillofac Surg., 69: 2305-12, 2011.

41. Leone, S.A., Edenfield, M.J., and Cohen, M.E.: Correlation of acute pericoronitis and the position of the mandibular third molar. Oral Surg Oral Med Oral Pathol., 62:245-49, 1986.

42. American Association of Oral and Maxillofacial Surgeons Task Force. AAOMS White Paper on Third Molar Data. American Association of Oral and Maxillofacial Surgery, 2007.

43. Sammartino, G., Tia, M., and Marenzi, G.: Use of autologous platelet-rich plasma (PRP) in periodontal defect treatment after extraction of impacted mandibular third molars. J Oral Maxillofac Surg., 63:766-73, 2005.

44. Sammartino, G., Tia, M., and Gentile, E.: Platelet-rich plasma and resorbable membrane for prevention of periodontal defects after deeply impacted lower third molar extraction. J Oral Maxillofac Surg., 67:2369-76, 2009.

45. Baslarli, O., Tumer, C., Ugur, O., and Vatankulu, B.: Evaluation of osteoblastic activity in extraction sockets treated with platelet-rich fibrin. Med Oral Patol Oral Cir Bucal., 20:111-118, 2015.

46. Eshghpour, M., Dastmalchi, P., Nekooei, A.H., and Nejat, A.: Effect of platelet-rich fibrin on frequency of alveolar osteitis following mandibular third molar surgery: A double-blinded randomized clinical trial. J Oral Maxillofac Surg., 72:1463-70, 2014.

47. Kan, K.W., Liu, J.K., and Lo, E.C.: Residual periodontal defects distal to the mandibular second molar 6-36 months after impacted third molar extraction. J Clin Periodontol., 29:1004-1011, 2002.

48. Simonacci, F., Bertozzi, N., Grieco, M.P., Grignaffini, E., and Raposio, E.: Procedure, applications, and outcomes of autologous fat grafting. Ann. Med. Surg., 20: 49–60, 2017.

49. Hosseini, M. and Shafiee, A.: Engineering bioactive scaffolds for skin regeneration. Small, 17(41), p.2101384, 2021.

50. Zhang, W. and Yelick, P.C.: Craniofacial tissue engineering. Cold Spring Harbor perspectives in medicine, 8(1), p.a025775, 2018.

51. Dohan, D.M., Choukroun, J., Diss, A., Dohan, S.L., Dohan, A.J., Mouhyi, J. and Gogly, B.: Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. Oral Surg, Oral Med, Oral Pathol, Oral Radiol, Endod, 101(3), 3744-50, 2006.

52. Choukroun, J., Diss, A., Simonpieri, A., Girard, M., Schoeffler, C., and Dohan, S. L.: Platelet rich fibrin (PRF): a second-generation platelet concentrate. Part IV: clinical effects on tissue healing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod., 101(3):56–60, 2006.

53. Gurbuzer, B., Pikdoken, L., and Tunah, M.: Scintigraphic evaluation of osteoblastic activity in extraction sockets treated with platelet-rich fibrin. J Oral Maxillofac Surg., 68:98-105, 2010.

54. Mai, C.T., Isenburg, J.L., Canfield, M.A., Meyer, R.E., Correa, A., Alverson, C.J., Lupo, P.J., Riehle-Colarusso, T., Cho, S.J., Aggarwal, D. and Kirby, R.S.: National population-based estimates for major birth defects, 2010–2014. Birth defects research, 111(18):1420-1435, 2019.

55. Trubelja, A., Kasper, F.K., Farach-Carson, M.C. and Harrington, D.A.: Bringing hydrogel-based craniofacial therapies to the clinic. Acta biomaterialia. 2021.