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EFFECT OF XENO-HYDROXYAPETITE BONE GRAFT ON THE HEALING OF UNFAVORABLE MANDIBULAR FRACTURES ON DOGS

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KEYWORDS

Mandibular Fractures; Xeno-Hydroxyapetite Bone Graft; Healing

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ABSTRACT

Introduction: Mandibular fractures are considered the most common facial bones fractures. Treating mandibular fractures is difficult due to probability of complications, and there is presently no consensus on the best therapy. **Aim:** This study aimed to evaluate the role of xeno-hydroxyapetite bone graft in the healing of unfavorable oblique mandibular fractures under rigid fixation. **Material and methods:** This experimental study included 18 healthy male dogs. Unilateral mandibular fractures were induced for all animals. Animals were divided equally into: Control group (I) where the fracture was fixed with reconstruction bone plate only. Study group (II) where the fracture was fixed with reconstruction bone plate and bone defect was filled with the xeno-hydroxyapetite bone graft. The bone defect site was examined radiographically, Histopathologically and histomorphometrically at 2, 4 and 8 weeks. **Results:** There was significant increase in bone density with increasing time in both groups. The analysis of histological sections revealed that the newly formed bony spicules in both groups were localized randomly and in discrete puncta within the graft material. Histomorphometry analysis suggests that the bovine bone graft showed well-organized and reconstructed bone similar to native bone tissues. **Conclusion:** Xeno-hydroxyapetite bone graft is a good choice for the jaw bone reconstruction and can reduce the healing time in the area of the fracture line. Xeno-hydroxyapetite bone graft can be satisfactorily used as grafts in the treatment of unfavorable mandibular fractures.

INTRODUCTION

Mandible fractures represent 25% of maxillofacial fractures. Violence is the primary factor in the causation of mandibular body fractures, which represent 11% to 36% of all mandible fractures. Numerous factors as the degree of displacement, the loss of soft and hard tissues, the health of the teeth, the talents of the treating physicians, and the availability of hospitals, all have an impact on mandibular fractures [1,2].

The presence of malocclusion is crucial in developing an appropriate surgical strategy because one of the goals of treatment is occlusion restoration [3]. Soft diet alone, closed reduction with maxillamandibular fixation, open reduction, and internal fixation are all options for treating mandibular fractures [4].

Various type of bone grafts, such as autografts, allografts, synthetic grafts have been used in clinical settings [5,6]. For bone restoration,

hydroxyapatite (HA) is frequently and successfully employed that made from natural ingredients such egg shell, coral, fish bone, coral, human bone and bovine bone [7,8].

Therefore, this study aimed to evaluate the role of xeno-hydroxyapetite bone graft in the healing of unfavorable oblique mandibular fractures under rigid fixation.

MATERIAL AND METHODS

Approval of the Research Ethical Committee of the Faculty of Dentistry, Suez Canal University (59/2017) was obtained before starting the study.

This study was conducted on 18 healthy male adult mongrel dogs weighted 10-17 kgs. The dogs were kept under hygienic conditions in animal house at Faculty of Veterinary Medicine, Suez Canal University where the experiments were performed.

The animals were divided equally into:

(I) **Control group**: where the fracture was fixed with reconstruction bone plate only.

(II) **Study group:** where the fracture was fixed with reconstruction bone plate and bone defect was filled with the Xeno-Hydroxyapatite bone graft that was prepared in the Faculty of Science, Suez Canal University under the supervision of professor Sabry El-Korashy.

A. Preoperative preparation:

The cages were sprayed with Diazinone (*Memphis Co., Egypt*) and dogs injected by Ivomec (*El Nasr Co., Egypt*) against parasitic infestations. IV injection of prophylactic antibiotic (*Flumox, EPICO Co., Egypt*) to each dog for one day before surgery and 12 hours fasting animals to prepare them for anesthesia.

B. Surgical preparation:

The surgical procedure was done under the effect of the general anesthesia with complete aseptic condition. IV cannula inserted in recurrent tarsal vein for anesthesia induction using sodium thiopental (*2.5% sol., EPICO Co., Egypt*) was given slowly till loss of pedal corneal reflexes, constriction of the pupils and development of shallow regular respiration.

Operative procedures:

The dog was fully draped except the operative site was scrubbed with Betadine (povidine iodine 2%, Nile, Egypt). Infiltration injection technique was applied in the submandibular region with (1ml) Mepivacaine HCL 2% with Levonordephrine 1:20000 (*Alexandria pharmaceutical Co., Egypt*) for hemostasis at the operative site. The incision was done in the submandibular region 1 cm below the inferior border of the mandible, then dissection was done till incising periosteum. 5 mm width defect was done from buccal cortex to lingual cortex with surgical fissure bur to create unfavorable fracture followed by using mallet and chisel till complete un- favorable fracture was created.

In control group: reduction of the unfavorable fracture and fixation by locking plates 2.3 mm (*produced by GLOBAL D – Fournisseur de materiel medical et chirurgical – Brignais France*) fixed with bicortical screws 11 mm using a drill on a straight hand piece with saline solution as a coolant.

In Study group: xeno-hydroxyapetite bone graft was filled in the created bone defect before fixation with reconstruction bone plate (lock type 2.3mm) (Fig. 1).

Then wound was irrigated with saline and closed using absorbable suture material Vicryl 3/0 (*Egysorb, Braided Polyglycolic acid, Taisier Medical, Egypt*).

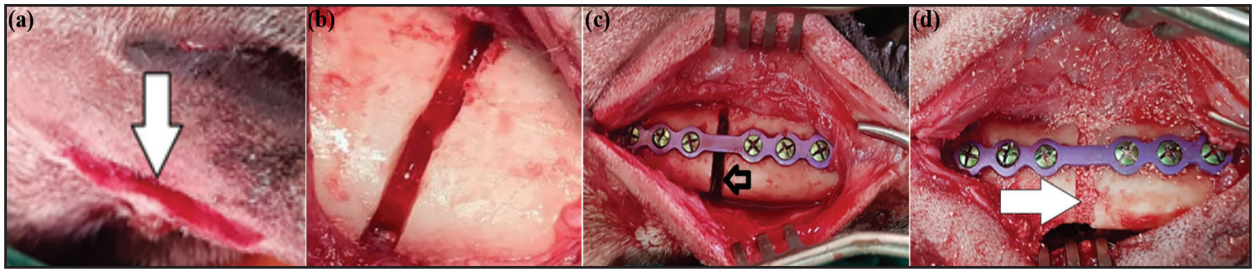

Fig. (1) Surgical procedure showing (a) submandibular incision (white arrow) (b) complete bone defect of the body of the mandible; (c) mandibular fracture fixation with the reconstruction plate in control group; (d) bone graft filling the bone defect reduced and fixed with reconstruction bone plate.

Post-operative care

For postoperative pain relief, IM injection of Diclofenac (*75mg, Novartis pharma.S.A.E, Egypt*) at the first 24 hours, and 1gm amoxicillin with sulbactam IV for four days to reduce infection. The animals were watched daily for signs of complications as infection or mobilization of the fractured mandible till the time of scarifications. The dogs were kept on soft diet for the first post-operative week, after the first week dogs were on regular diet.

Post-operative follow up

The animals were scarified using overdose IV of thiopentone sodium (3 from each group) at 2, 4, and 8 weeks (W) post-operatively. After scarifying the animals, the bone defect site was examined radiographically, histopathology and histomorphometrically.

Radiological assessment

Which was done by reading carefully each CBCT for each mandible and ROI (region of interest) which is the data set identified for determining the measures of bony defect of each dog in each group with in the variable intervals mentioned before was recorded by measuring the density values of pixels with in the marked area, and measures the minimum, maximum , average and standard deviation density values with in a region ,then , all gathered data were statistically analyzed.

Histomorphometric analysis

Image J software , National Institute of Health, Bethesda, MD, USA) was used to perform a quantitative analysis of the bone area, graft area, and bone marrow spaces in the control and study group. Plug-in simply includes bone skeleton in BoneJ was used to analyze the bone area, graft material, and bone marrow spaces in different groups. The area/volume fraction is used to calculate the percentage of bone in an image when compared to the entire picture. It counts all the foreground voxels in the picture, which it assumes are bones and compares them to the total number of voxels. GraphPad Prism was used to analyze the mean statistical difference using one-way analysis of variance (ANOVA) and Tukey's post hoc tests for pair-wise comparisons. *p 0.05, **p 0.01, and ***p 0.001; data are shown as mean standard error of mean (SEM) [9].

RESULTS

I. Comparison of bone density measures among observation times:

There was a prominent change among observation times for control group (P<0.001). The mean bone density was significantly increased after

4 weeks (89.700±4.300 Hounsfield) using Meyer CBCT machine model ss-X9010DPro-3DE with image voxel 40-80 µm using windows 10 operating system and 8 W (190.667±11.930) compared to 2 W with ratios 691.7% and 1582.78% respectively (Fig. 2). There was a prominent change among observation times for study group (P<0.001).The mean bone density was significantly increased after 4 W (164.00±21.656) and 8 W (239.33±15.144) compared to 2 W (59.67± 3.055) with ratios about 179.87% and 301.08% respectively (Fig. 3).

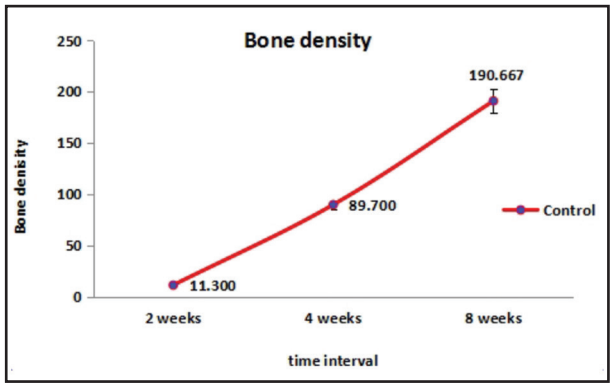

Fig. (2) Comparison of bone density measures among study observation times for control group. Error bars represent confidence interval.

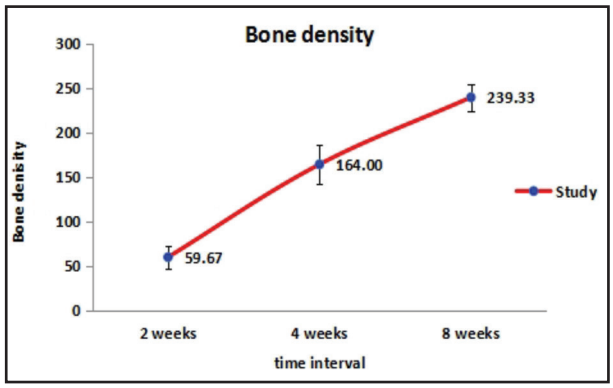

Fig. (2) Comparison of bone density measures among study observation times for study group. Error bars represent confidence interval.

II. Comparison of bone density between groups at different observation times

For all observation times, study group showed significant higher bone density than control group (P<0.001). After 2 W, there was a significant difference between groups (p<0.001). The mean of bone density showed higher density values (59.667±3.055) of study group than control group (11.300±1.473)**.** After 4 W, the mean of bone density measures in study group showed significant higher density values (164.00±21.656) than control group (89.700±4.300). After 8 W, the mean of bone density measures in study group showed highly significant density values (239.333±11.930) than control group (190.667±15.144) **(Table 1).**

Table (1) *Comparison between control and study groups at the same period.*

Group		Mean	SD	Mean difference	T	Sign.
2 weeks	Control	11.300	1.473	48.37	24.70	<0.001**
	Study	59.667	3.055			
4 weeks	Control	89.700	4.300	74.30	5.829	0.004**
	Study	164.000	21.656			
8 weeks	Control	190.667	11.930	48.67	4.372	0.012**
	Study	239.333	15.144			

III. Histological Findings

The control group; after 2 weeks; showed that the defect was filled with granulation and fibrous collagenous tissue. Connective tissue matrix was filled with cellular elements and blood capillaries with hemorrhage and few inflammatory cells. Newly radiating bone matrix connected with the original bone of the socket. Osteoclasts present at the bone surface margin and bone marrow spaces. While, the study group showed that the defect was filled with

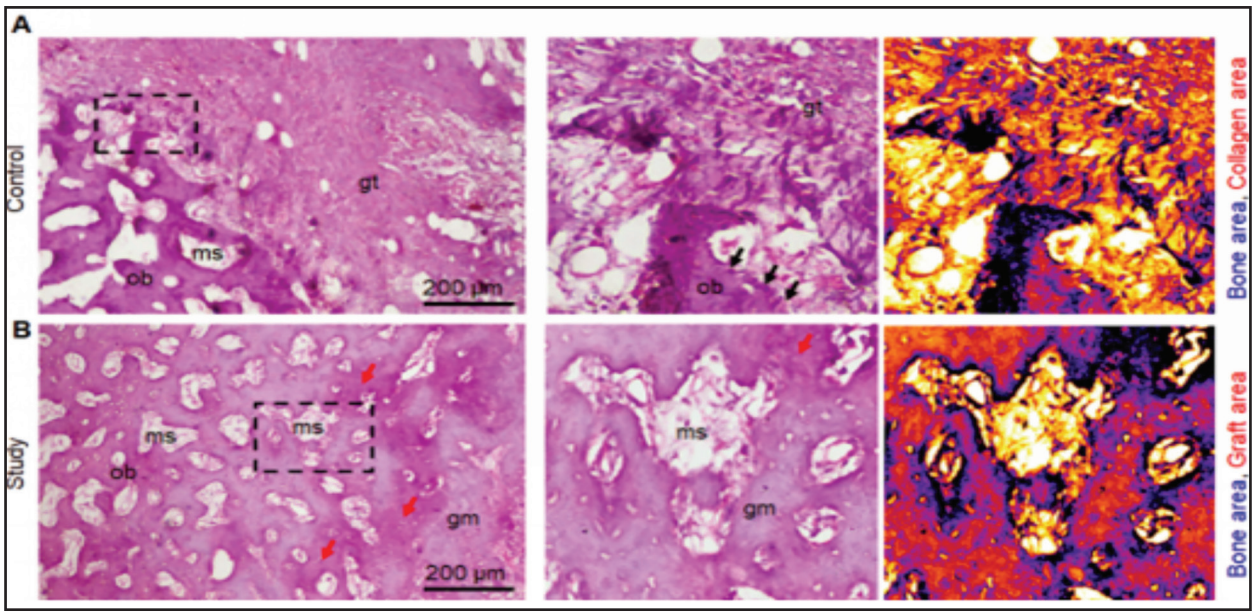

Fig. (4) Histological images were stained with H&E of the control and study group after 2 weeks. (A) The Control group showed the socket filled with collagenous fibrous (arrowheads) granulation tissue (gt) contained vascular proliferation and inflammatory cells. Osteoclasts (black arrows) were located in their howships lacunae at the margin of original bone (ob) and bone marrow spaces (ms). Discrete radiating interconnecting bony patches were observed (rectangular). (B) The study group showed interlacing newly formed bone trabeculae of woven bone with wide medullary spaces. The bony defect was filled with graft material (gm) in which newly developed bone spicules were randomly organized (red arrows). Bone skeleton analysis by image J is shown at the right (blue color indicates the bone area while red/yellow color indicates the collagen tissue in the control group or graft materials in the study group). (Scale bar= 200 μm).

graft material in which developed bone spicules were organized. Wide medullary spaces filled with bone marrow, vascular tissue and few inflammatory infiltrations were observed within the graft material. Immature osteoblasts and osteocytes appeared close to the bone marrow spaces boundaries (Fig.4).

The control group after 8 weeks revealed complete defect obliteration by a thick interconnecting trabecular bone characterized by mature osteocytes in their lacuna with regularly marrow spaces. A few fibrous tissues distributed within the socket. The area of compact bone was observed with the appearance of the Haversian system. In the study group revealed the bone defect entirely occupied by a thick interconnecting trabecular bone of different thicknesses, characterized by the presence of mature osteocytes in their lacuna and parallel basophilic matrix (Fig.5).

IV. Histomorphometry analysis:

The analysis showed that new bony formation was significantly increased after 8 weeks (78.35%±5.5) compared to 2 weeks (7.927%±0.42) and 4 weeks (40.234%±3.5). The area of graft material was significantly decreased after 8 weeks (5.631%±0.3) when compared with 2 weeks (64.258%±4.5) an d 4 W (35.681%±2.5). While for the bone marrow spaces, there is a slight decrease in their areas after 8 weeks. A significant difference between both groups after 4 weeks (17.413%±2.5 for the control, 40.234%±3.5 for the study) and 8 W, (38.133%±2,1 for control, 78.351%±5.5) (Fig.6).

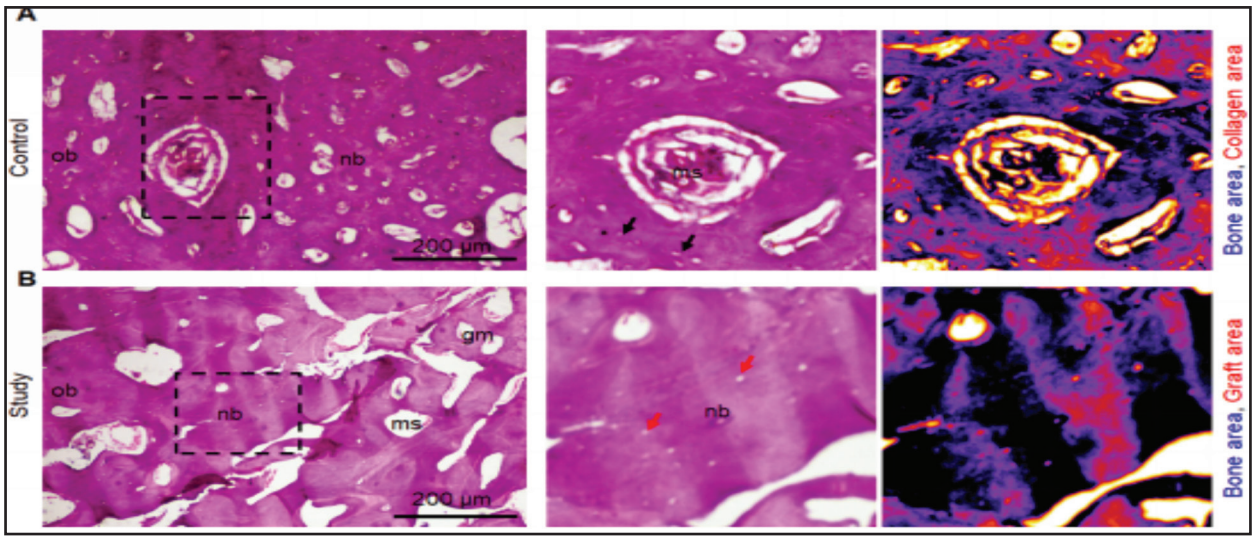

Fig. (5) Histological images were stained with H&E of the control and study groups after 8 W. (**A**) Control group showed the socket filled by a thick interconnecting trabecular bone of varied thicknesses of new bone (nb) with mature osteocytes (black arrows) in their lacuna. (B) Study group showed bone defect entirely occupied by a thick interconnecting trabecular bone of different thicknesses of new bone (nb) with mature osteocytes in their lacuna (red arrows). Bone skeleton analysis by image J is shown at the right (blue color indicates the bone area while red/yellow color indicates the collagen tissue in the control group or graft materials in the study group). (Scale bar= 200 μm).

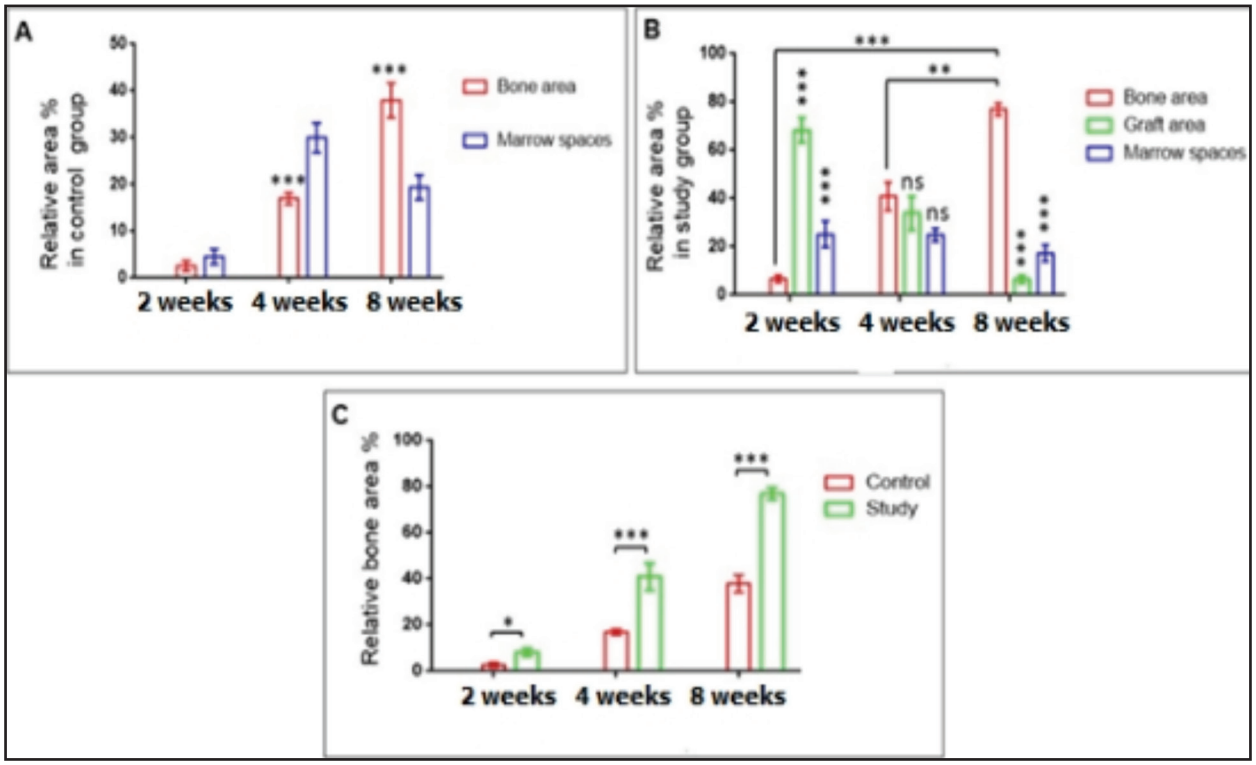

Fig. (6) Histomorphometry analysis of control and study groups at different time zone. Quantitative analysis of the bone area, graft area, and bone marrow spaces area in the control group (**A**) and the study group (**B**). Quantitative analysis of the bone area in control and study groups (**C**).

DISCUSSION

Surgical techniques combining the use of bone grafts and/or biomaterials have been developed to recover the normal anatomical shape and allow functional and physiological oral rehabilitation with the use of implants and/or conventional prosthesis, despite the fact that bone tissue has a remarkable regeneration potential to restore its original structure and mechanical properties [10,11].

Hydroxyapatite (HA) has a chemical composition and crystalline structure like bone, is the most frequently utilized bioceramics material for bone grafting [12].

In the present study, the fractures were fixed by two different procedures; in the control group, the fractures were fixed with reconstruction bone plate only, while in the study group, the fractures were fixed with reconstruction bone plate and bone defect were filled with the bovine xeno-Hydroxyapetite bone graft.

The bone density measures were compared among the current study observation times for the control and study groups. The mean bone density was significantly increased after 4 & 8 W. The study group showed a significant increase in the bone density more than the increase the control group. This means that fixing the mandibular fractures with reconstruction bone plate and filling the bone defect with the xeno-Hydroxyapetite bone graft is better as an increase in opacity indicated an increase in bone formation in the defect area [13]. This could be attributed to the fact that HA as a natural structure has low immunogenicity with low inflammatory response and good integration of HA [14]. In addition, it was reported that HA significantly prevents loss of bone after extraction in animal and human trials [15]. Also, **Xu *et al.*** [16] showed short fibers that contained BMP-2 were coated on HA samples using porous samples. They concluded that fiber coating is an efficacious method to improve both mechanical and osteogenic characteristics of HA samples.

The histological evaluation of the present study was in agreement with **Manjubala, *et al.*** [17] reported that complete Haversian system was observed after 12 weeks in a dog model.

Voor *et al.* [18] investigated the effects of adding decalcified xenograft bone to Hydroxyapatite cement (HAC) resorption, on new bone formation, and strength over ten weeks in an established animal model. Adding xeno graft to HAC creates a bioactive composite that is more rapidly incorporated, resorbed, and replaced by new bone. The presence of xenograft particles creates a vigorous inflammatory response, but there may be some benefit to the resorption rate of the HAC component of the xenograft bone particles from young pigs due to the infiltration of cells. This volumetric inclusion of rapidly resorbed bone graft does not compromise the initial indentation strength of the filled defect relative to normal cancellous bone.

In contrast to our study, **Rahimnia *et al.*** [13] compared and evaluated the synthetic hydroxyapatite with xenograft and commercial hydroxyapatite for bone repair and reconstruction, revealed that the xenograft and commercial synthesis HA groups showed a small quantity of ossification at the edges of natural bone at 4 weeks.

Clinically, this commercial xenograft has proven to be a valuable bone substitute material providing good quality of new bone and promising long-term survival rates following dental surgery [19].

In our histomorphometry analysis of study groups revealed a new bony formation was significantly increased after 8 W (78%) compared to other group at 2 W (6%) and 4 W (40%). These results were in disagreement with **Rahimnia *et al.*** [13] where the

histomorphometric analysis showed that the rate of new bone formation in the repaired samples in the synthesised synthetic,commercial synthetic, xenograft, and control group at 4 W was 17.4, 6.6, 7.1, and 0.8%, respectively. In addition, the amount of new bone was greater in the repaired defects in the synthesised HA samples during 8 and 12 weeks (35.7 and 55.5%, respectively), which were significantly different than the repair process of other groups. At 8 W post-surgery, xenograft and commercial synthesis HA groups, had cell penetration and bone formation were greater than at 4 W. The remainder of the sample was covered with connective tissue. The rate of ossification at 8 weeks from the margin to the centre in the synthesized HA group was higher than that of the xenograft HA, commercial synthesis HA. **Sousa *et al.*** [20] conducted a histomorphometric study for evaluation of osteoconduction and bioresorption of bone allograft versus anorganic bovine xenograft bone. The results revealed that the efficiency of allograft in relation to xenografts in the new bone formation, although both materials have the osteoconductive property.

Another study conducted by **da Silva Brum** [21] investigated the tissue behaviours of two distinct types of biomaterials in sub-critical bone defects in rat calvaria which were; synthetic nano-hydroxyapatite/ beta-tricalcium phosphate composite and xenograft bone. The findings of that study also showed that combining of different biomaterials (nano-HA with-TCP) resulted in a positive outcome than using the xenograft bone alone.

Furthermore, the xenograft bovine bone's features of delayed reabsorption rate and strong resistance are responsible for the smaller bone development in the bone defect. This lacks the effect on HA mineralization has previously been noted [115,150].

CONCLUSIONS

- The xeno-hydroxyapetite bone graft is a good choice for the jaw bone reconstruction and also useful in decreasing the healing time in the area of the fracture line.
- Xeno-hydroxyapetite bone graft presents a greater osteoconductivity and faster bioresorption. Also xeno-hydroxyapetite bone graft can be satisfactorily used as grafts in the treatment of unfavorable mandibular fractures.
- The histomorphometry analysis suggests that the bovine bone graft showed well-organized and reconstructed bone similar to native bone tissues.

REFERENCES

1. Olate S, Assis A, Pozzer L, Cavalieri-Pereira L, Asprino L, Moraes M. Pattern and treatment of mandible body fracture. Int J Burns Trauma. 2013; 3: 164–168.
2. Rahpeyma A, Khajehahmadi S, Mehni SB. Treatment of Mandibular Fractures by Two Perpendicular Mini-Plates. Iran J Otorhinolaryngol. 2014; 26: 31–36.
3. Prein J. Manual of Internal Fixation in the Cranio-facial Skeleton. Berlin, Germany, Springer-Verlag, 1998; 45-56.
4. Kellman RM. Maxillofacial trauma. In Cumming's Otolaryngology: Head and Neck Surgery, 5th ed. 2010 Pp 111-156.
5. Abreu M, Viegas VN, Ibrahim D, Valiati R, Heitz C, Silva D. Treatment of comminuted mandibular fractures: Med Oral Patol Oral Cir Bucal. 2009;14:247-251.
6. Zipfel GJ, Guiot BH, Fessler RG. Bone grafting: Neurosurg Focus. 2003;14:22-25.
7. Salmasi S, Nayyer L, Seifalian AM, Blunn GW. Nanohydroxyapatite Effect on the Degradation, Osteoconduction and Mechanical Properties of Polymeric Bone Tissue Engineered Scaffolds. Open Orthop J. 2016; 10: 900–919.

8. Kattimani S, Srinivas P, Krishna L. Biograft Block Hydroxyapatite: A Ray of Hope in the Reconstruction of Maxillofacial Defects. 2016; 27: 247–252.
9. Suvarna KS, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques (8th ed.) Cambridge, MA: Elsevier,2019.
10. Sheikh Z, Sima C, Glogauer M. Bone replacement materials and techniques used for achieving vertical alveolar bone augmentation. Materials,2015; 8(6), 2953-2993.
11. Wang W, Yeung KW. Bone grafts and biomaterials substitutes for bone defect repair: A review. Bioactive materials, 2017; 2(4), 224-247.
12. Dewi AH, Ana ID. The use of hydroxyapatite bone substitute grafting for alveolar ridge preservation, sinus augmentation, and periodontal bone defect: a systematic review. Heliyon,2011; 4(10), e00884.
13. Rahimnia A, Hesarikia H, Karami S, Kaviani K. Evaluation and comparison of synthesised hydroxyapatite in bone regeneration: As an in vivo study. Journal of Taibah University Medical Sciences, 2021; 16(6), 878-886.
14. Elsharkawy LF, Elprince NH, Sweedan AO. Evaluation of immediate implant placement with a modified socket shield technique using xenogenic bone gratf (a randomized controlled clinical trial). ADJ, 2021; 46(3), 29-36.
15. McPherson R, Vickers PG, Slater GL. Bone grafting with coralline hydroxyapatite. EC Dent Sci. 2019; 18: 2413-2423.
16. Xu T, Sheng L, He L, Duan K. Enhanced osteogenesis of hydroxyapatite scaffolds by coating with BMP-2-loaded short polylactide nanofiber: a new drug loading method for porous scaffolds. Reg Biomate, 2020; 7(1), 91-98.
17. Manjubala I, Sastry TP, Kumar RS. Bone in-growth induced by biphasic calcium phosphate ceramic in femoral defect of dogs. J Biomate Appl. 2005; 19(4): 341-360.
18. Voor MJ, Yoder EM, Burden RL Jr. Xenograft bone inclusion improves incorporation of hydroxyapatite cement into cancellous defects. J Orthop Trauma. 2011; 25(8):483-487.
19. Uzbek UH, Rahman SA, Alam MK, Gillani SW. Bone forming potential of an-organic bovine bone graft: A cone beam CT study. JCDR. 2014; 8(12), ZC73.
20. Sousa SB, Castro-Silva I, Lenharo A, Granjeiro JM. Osteoconduction and bioresorption of bone allograft versus an organic bovine bone xenograft: a histomorphometric study in humans. JBBTE. 2023; 18:85-95.
21. Da Silva Brum I, Frigo L, Lana Devita R, da Silva Pires JL, de Carvalho JJ. Histomorphometric, immunohistochemical, ultrastructural characterization of a nano-hydroxyapatite/beta-tricalcium phosphate composite and a bone xenograft in sub-critical size bone defect in rat calvaria. Material. 2020; 13(20): 4598.