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ROLE OF NANO-BIOCERAMIC BONE GRAFT ON THE HEALING PROCESS OF UNFAVORABLE MANDIBULAR ANGLE FRACTURE (AN EXPERIMENTAL STUDY)

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ABSTRACT

Introduction: The most frequent fracture is a mandibular angle fracture, which is challenging to treat because there is no established universal technique. So, for diverse implant systems, different kinds of plates have been developed. **Aim:** This study aimed to evaluate of nano bio-ceramic bone graft with 3D bone plate on the healing of unfavorable mandibular angle fracture. **Material and methods:** This study included 18 male adult mongrel dogs, animals were divided into (9 of each): study group (I) where the angle fracture treated by 3 dimension bone plate with Nano bio glass bone graft. Control group (II) where the angle fracture treated by 3 dimension bone plate only. Noncritical size bone defects (3 mm diameter) were created in the mandibular angle of the dogs. Bone density was measured in the vicinity of the fracture line using the cone beam (CBCT). **Results:** Dogs were euthanized at 3, 6 and 12 weeks postoperatively, six dogs at a time, three dogs from each group. All of the 18 fracture line healed uneventfully. Soft tissue healing normally proceeded without any signs of infection. Post-operative assessment of the current study revealed no obvious complications; neither post-operative infection nor wound dehiscence was detected in any case. The synthetic Nano bio glass bone graft was bio compatible and non- allergic. CBCT showed that measurements of the bone density reading were increased in study group more than control group throughout showing highly significantly reading after 12 weeks. The measurements of the bone density were higher in the study group than control group throughout the whole study intervals. **Conclusion:** 3D Titanium plates have superior biomechanical properties and biocompatibility when used in treatment of fractures of the angle. Nano bio glass bone graft is a compatible material and doesn't interfere with the healing process of fracture filed.

INTRODUCTION

One of the most frequent injuries to the facial skeleton is a mandibular fracture. It has been estimated that between 40 and 62% of all facial fractures are caused by it. To restore the patient to their pre-injury condition of function and appearance should be the aim of treating mandible fractures(1). One of the most common locations for lower jaw fractures is the mandibular angle, which accounts for 20% to 36% of all mandibular fractures (2).

In recent years, mandibular fracture treatment methods have undergone a major evolution. These methods include wire osteosynthesis, open reduction with stiff internal fixation, and either adaptive miniplate fixation or closed reduction with maxillomandibular fixation (MMF) (3).

Angle fractures continue to rank among the most challenging and unpredictable fractures to treat when compared to those in other parts of the mandible, despite numerous advancements in internal fixation. Many investigations on the management of mandibular angle fractures have revealed that no single strategy has been demonstrated to be optimum, and that the management of mandibular angle fractures is still conceptually debatable (4).

Three-dimensional (3D) miniplates were created as a result of the drawbacks of hard fixation (compression and reconstruction plates) and semi-rigid fixation (standard mini plate). Conceptually, the shape of 3D strut plates enables stability in three dimensions, resistance to torque forces, and malleability while maintaining a low profile (5). The basis of 3D plating systems is the idea that support can be obtained through geometrically stable design. With its high resistance to twisting forces, the quadrangle form of the plate ensures good stability in three dimensions of fracture (6).

By promoting osteoblast migration, proliferation, and differentiation, repair and regeneration processes can be accelerated for swift function restoration (7). Therefore, this study aimed to evaluate of nano bio-ceramic bone graft with 3D bone plate on the healing of unfavorable mandibular angle fracture.

MATERIAL AND METHODS

Eighteen mature male dogs, weighing between weighing 15–20 kg, their ages ranged from 1-2 years. The dogs were given cooked meat and water while being kept under clinical surveillance for 3 weeks prior to surgery. The animals were kept in separate cages, and given the freedom to thrive in a healthy environment. All animals subjected to the surgical procedures at the Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Suez Canal University.

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

Unilateral angle fracture was induced, these animals divided into two equal groups (9 of each).

Group I (study group): The angle fracture treated by 3 dimension bone plate with Nano bio glass bone graft.

Group II (control group): The angle fracture treated by 3 dimension bone plate only.

Preoperative preparation:

The cages were sprayed with 6/1000 ml diazinone[1] and dogs were injected by Ivermectine[2] (0.1mg/kg b.wt S/C). Animals were fasten for 12 hr. before operation. Prophylactic antibiotic-1gm amoxicillin IV to each dog for one day before surgery.

Synthetic bio glass with a chemical composition: $CaO\text{-}SiO_2\text{-}Na_2O\text{-}P_2O_5$=46.1-26.9-34.4-2.6 ml% was prepared by sol gel method reported by **Xia** (8).

Surgical protocol:

The operation performed under general anesthesia as the following protocol:

A. All animals were premeditated preoperatively by subcutaneous administration of Atropine sulphate[3] 0.005mg/kg body weight 10-30 minutes prior to surgery.

B. Cannulation of the cephalic vein using 20 gauges; IV cannula.

1. Diazinone, Memphis Co. for pharmaceutical and chemical industry. Cairo. Egypt.

2. Ivomec El Nasr Co. for pharmaceutical, Giza, Egypt.

3. Memphis Co., Cairo.

C. Dogs were administered Neurazine[4] IM ampoule 25 mg/Kg (Chlopromazine HCL).

D. Induction of anesthesia by IV sodium thiopental[5] 2.5% solution 20-30 mg/Kg given slowly till loss of reflexes, constriction of the pupils and shallow regular respiration.

Surgical Operation:

The animals were placed in a lateral recumbent position. The surgical sites were painted with Betadine[6], followed by local anesthesia with vasopressor[7]. With the No. 3 Bard Parker scalpel handle and No. 10 disposable blade, an additional oral incision is made. A complete defect was made using a low speed motor, a surgical fissure bur that is externally chilled with a syringe of saline solution, and a surgical chisel and mallet that are positioned perpendicular to the mandibular angle to make unfavorable defect (Figure 1).

In Group I: the angle fracture treated by 3 dimension bone plate with Nano bio glass bone graft. Nano bio-glass bone graft mixed with blood gathered from the fracture line and put the mix inside the defect. The 3D plate was positioned in accordance with Farmand and Dupoirieux's instructions, with the horizontal bars parallel to the fracture line and the vertical bars perpendicular. Two 2.0 mini screws were used to fix the plate's lower border first, and two 2.0 mini screws were used to repair the plate's top border's two peripheral holes using a drill on a straight hand piece that was chilled externally with saline solution.

In Group II: Double square 3D titanium plates of 1.0 mm thickness (3 x 2 holes joined by vertical struts) that are fastened to the fracture using 2.0 mm titanium screws that are 7 mm long were used to treat the fracture (Figure 2).

Saline solution was used to irrigate the wound and closed it using absorbable suture 3-0 suture material Vicryl*. The skin was closed using non absorbable silk 3-0 suture material.

Our study aimed to evaluate of nano bio-ceramic bone graft with 3D bone plate on the healing of unfavorable mandibular angle fracture, so we induced this fracture to investigate the occurrence of osteoinduction or osteocondution with noncritical size bone defects (3 mm diameter) were created in the mandibular angle of the dogs.

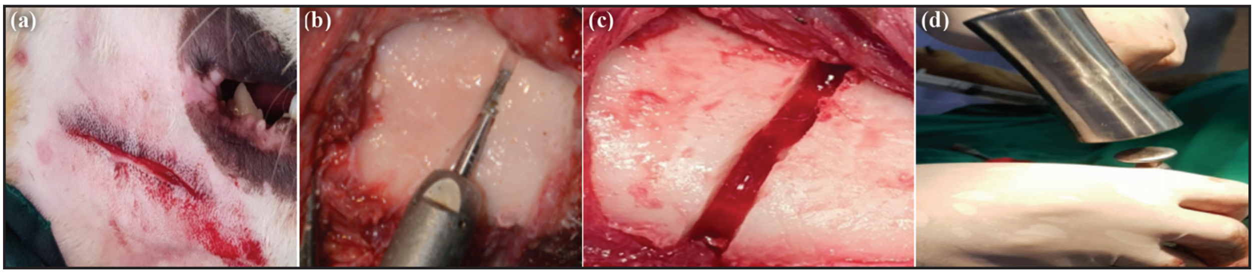

Fig. (1) Surgical procedure showing (a) surgical site was painted with Betadine and extra oral incision in skin; (b) surgical fissure bur for creating mandibular angle defect; (c) complete bone defect in mandibular body; (d) using chisel and mallet to make unfavorable defect

4. *Manufacture by Misr Co. Egypt.*

5. *FARCOPENTAL vial (500 mg thiopental Na in 20ml solvent) Pharco pharmaceuticals, Alexanderia, Egypt.*

6. *Betadine antiseptic solution (povidine iodine 2%), Nile, Egypt.*

7. *Mepecaine L (Mepivacaine HCL 2% with Levonordefrin 1: 20000), Alexandria pharmaceutical Co, EGYPT.*

8. *Vicryle (TRUGLYDE TM) manufactured by SUTURES, INDIA, PVT, LTD.*

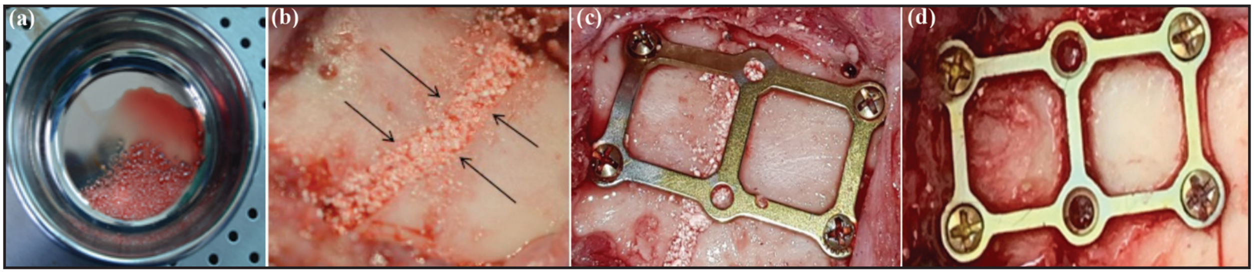

Fig. (2) Surgical procedure in group I showing (a) mixed Nano bio glass with blood; (b) the bone defect completely filled with Nano bio glass (arrow); (C) fracture fixation with a 3D titanium plate and nano bio glass occupied the fracture line (study group). (d) Fixation with a 3D titanium plate only (control group)

Post-operative follow up

For postoperative pain relief, Voltaren[9] was given IM during the first 24 hours, and 1gm amoxicillin[10] IV for 4 days. The animals were examined daily for signs of illnesses. All dogs underwent clinical wound dehiscence, infection, swelling, and edoema checks following surgery. All of the animals were given soft consistency food to eat. Ten days after surgery, the skin stitches were removed.

The quantitative evaluation:

Leica Qwin 500 image analyzer computer system was used to do the quantitative histomorphometric evaluation (England). The image analyzer was made out of an Olympus BX40 microscope, a colored video camera, a colored monitor, and an IBM computer's hard drive (Olympus, Japan). The image analyzer was first calibrated automatically to transform the measurement units (pixels) produced by the image analyzer into actual micrometer units.

Radiographic assessment:

(a) **Cone beam volumetric imaging:** Following surgery, the bone density of the produced bone in the surgical defect was measured at intervals of 3, 6, and 12 weeks using the ON DEMAND viewer software of Sirona cone-beam volumetric imaging.

(b) **Density measurement:** The ROI (Region Of Interest) method was used to measure surgical flaws since it measures the minimum, maximum, average, and standard deviation of the density values inside a region.

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

The present study showed bone density measures for control group as mean value ±SD after 3 weeks postoperatively was (25.59+21.02) and maximum reading of bone density was (47.08). While the mean value after 6 weeks was (30.00 + 23.63) and maximum reading of bone density was (55.90). The mean value ±SD after 12 weeks was (41.33 + 41.40) and maximum reading of bone density was (88.00) (Pvalue <0.001) (Table 1). In the study group, the mean value±SD after 3 weeks postoperatively was (60.50±30.50) and maximum reading of bone density was (91.30). The mean value after 6 weeks was (145.56±56.96) and maximum

9. Diclofenac sodium, manufactured by, Novartis pharma.S.A.E Cairo, Egypt.

10. Flumox 1gm, manufacture by Eipico Co reading of bone density was (201.80). The mean value ±SD after 12 weeks was (192.36±23.00) and maximum reading of bone density was (215.40) (P-value <0.001) (Table 2).

Table (1) *Descriptive statistics of bone density measures for control group*

	Mean	Std. Deviation	Median	Minimum	Maximum
3 weeks	25.59	21.02	23	6.00	47.80
6 weeks	30.00	23.63	24.5	9.60	55.90
12 weeks	41.33	41.40	27	9.00	88.00

Table (2) *Descriptive statistics of bone density measures for study group*

	Mean	Std. Deviation	Median	Minimum	Maximum
3 weeks	60.50	30.50	59.90	30.30	91.30
6 weeks	145.56	56.96	147.00	87.90	201.80
12 weeks	192.36	23.00	192.30	169.40	215.40

Comparison of bone density measures among study observation times for control group. There was no significant difference in bone density among observation times for control group (P=.810) (Figure 3). Multiple comparisons (post hoc Bonferroni test) of bone density measures between each 2 observation times for control group (Table 3).

Table (3) *Multiple comparisons of bone density measures between each 2 observation times for control group:*

Observation times	Bonferroni test (p value)
3 weeks-6weeks	1.00
3 weeks-12 weeks	1.00
6 weeks-12 weeks	1.00

** p is significant at 5%.*

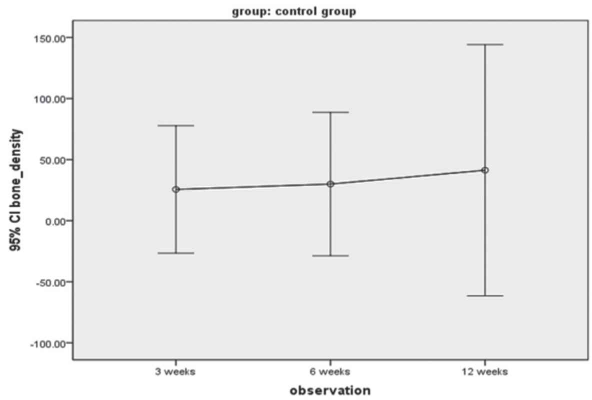

Fig. (3) Comparison of bone density measures among study observation times for control group. Error bars represent confidence interval.

Regarding comparison of bone density measures among study observation times for study group, there was a significant difference among observation for study group (P=.018) (Figure 4). Multiple comparisons (post hoc Bonferroni test) of bone density measures between each 2 observation times for study group. There was a significant difference between 3 and 12 W. No significant difference between 3 and 6 W or between 6 and 12 W. The mean bone density measures after 12 weeks (192.36±23.00) significantly increased (p=.020) compared to 3 weeks (60.50±30.50) (Table 4).

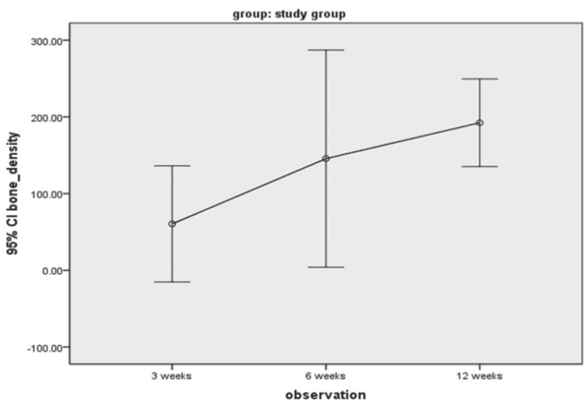

Fig. (4) Comparison of bone density measures among study observation times for study group. Error bars represent confidence interval

Table (4) *Multiple comparisons of bone density measures between each 2-observation times for study group*

Observation times	Bonferroni test (p value)
3 weeks-6weeks	.117
3 weeks-12 weeks	.020*
6 weeks-12 weeks	.59

** p is significant at 5%.*

After 3 W, no significant difference in bone density between groups was observed. After 6 and 12 W, Study group showed significant higher bone density than control group (Independent samples t-test, P=.032 for 6 Wand p=.005 for 12 W). After 6 W, there was a significant difference between groups (p=.032). The mean of bone density measures in study group showed higher density values (145.56±56.96) than that in control group (30.00±23.63). After 12 W, there was a significant difference between groups (p=.005). The mean of bone density measures in study group showed higher density values (192.36±23.00) than that in control group (41.33±41.40) (Figure 5).

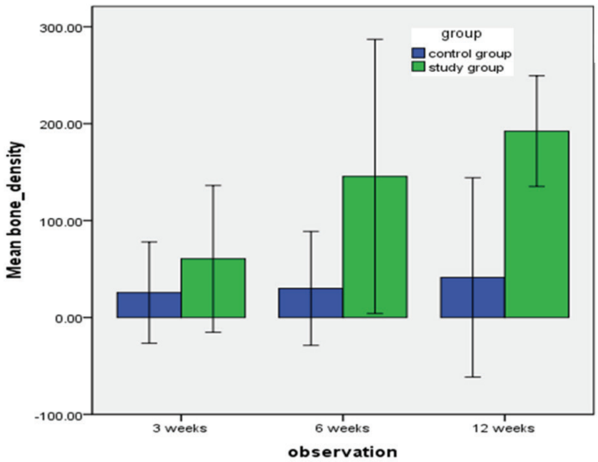

Fig. (5) Comparison of bone density measures between two groups at different observation times.

Regarding comparison of bone area fraction between groups at different observation times. At 3 weeks, 6 weeks and 12 weeks, there was a significant difference in bone area fraction between groups. Study group showed significant higher bone area fraction than control group (P=.030 for 3 W, p=.003 for 6 W, and p=.001 for 12 W). After 6 W, there was a significant difference between groups (p=.030). The mean of bone area fraction in study group showed higher density values (42.33±10.70) than that in control group (16.62±8.34). After 6 W, there was a significant difference between groups (p=.003). The mean of bone area fraction in study group showed higher density values (93.38±5.12) than that in control group (46.33±11.78). After 12 W, there was a significant difference between groups (p=.0051). The mean of bone area fraction in study group showed higher density values (98.49±.90) than that in control group (83.04±3.00) (Table 5).

Table (5) *Comparison of bone area fraction between two groups at different observation times*

	3 weeks		6 weeks		12 weeks	
	X	*SD*	*X*	*SD*	X	SD
Control group	16.62	8.34	46.33	11.78	83.04	3.00
Study group	42.33	10.70	93.38	5.12	98.49	.90
Independent t-test (p value)	.030*		.003*		.001*	

*X: mean, SD, standard deviation, * p is significant at 5*

DISCUSSION

The most frequent fracture is a mandibular angle fracture, which is challenging to treat because there is no established universal technique for doing so. Several types of implants (plates) have been designed for various implant systems, each claiming to be superior to the others in terms of stability and problems [3].

In agreement with the current study, **Wittenberg**[9] reported that in the treatment of mandibular angle fractures, the advantage of speedy reduced time for transoral application of three-dimensional titanium manipulators is confirmed by the current study. After plate fixation, it was discovered that the fragmented fragments were stable.

In the current study, Mandibular angle fractures can be effectively treated with 3D titanium miniplates, and the outcomes are consistent with those described by **Farmand** [10] reported that with a three-dimensional plate system, considerable stability against traction forces and torsion forces is achieved. **Wittenberg *et al.*** [11] reported that mandibular angle fractures may be adequately fixed with a three-dimensional plating system.

A study by **Choi *et al.***[12], who found that two non-compressive miniplates may effectively stabilise a mandibular angle fracture and that is in agreement with the current study, support the fixation of a 4-holed square plate for mandibular angle fracture.

To diagnose a mandibular angle fracture, a radiologic exam is required. In the first assessment of suspected mandibular angle fractures, conventional radiographs like OPG, lateral skull view, posterior anterior views, conventional CT, and CBCT are helpful. CT or cone beam CT should be used when OPG cannot clearly demonstrate the degree of displacement, type of fracture, or degree of commination [13].

Conventional histological staining, which uses a number of established techniques, can be used to examine the behaviour of bone substitute materials in animal trials and from grafted sites in people. They offer data on the graft's microstructure with regard to integration or osteogenesis, but not on the variables that influence these processes [14].

Regeneration of the bone tissue that has been damaged by diseases or trauma is the fundamental objective of treating bone problems. In addition to autogenous, xenogenic, and allogenic bone grafts, and alloplastic materials, guided bone regeneration techniques have also been used frequently. Ideal candidates would have good osteoinductive potential, minimal inflammatory reactions, rapid vascularization, affinity with host tissues, easy accessibility, and should be affordable [15].

According to **Moore *et al.*** [16] revealed that a bioactive glass granules have recently been produced and are more quickly reabsorbed than hydroxyapatite, enabling for considerably more rapid new bone growth in the healing of bone defects., and that agrees with the current study. Osteoblasts have a disordered dorsal surface, many cytoplasmic projections, pseudopodia, a compact appearance, and strong cell membrane activity. Thus, bioactivity should be seen as essential for the creation of novel platforms for bone tissue bioengineering due to the adhesion and intimate contact between osteoblasts. Its use in the medical and dental fields has stimulated research on the viability of implant coating, in order to improve biologic fixation and also as a carrier of osteoinductive substances, such as bone morphogenetic proteins. This is because of its excellent biocompatibility and its physical and chemical properties [16].

The results of the current study regarding new bone formation found that the study group that used nano-bio glass showed more new bone formation than the control group. The advantage of the bio glass group over the control group in enhancing more new bone formation found in the results of the current study came in agreement with the study held by **Tadjoedin *et al.*** [17] **and Cordioli *et al.*** [18] who used bioactive glass for augmentation of the maxillary sinus floor and observed that in particles close to the sinus floor membrane, bone tissue was formed. Although in the current study the quantity of bone tissue produced in particles close to the soft tissue was not compared to particles close to the dog's bone.

CONCLUSION

- 3D Titanium plates have superior biomechanical properties and biocompatibility when used in treatment of fractures of the angle.
- Nano bio glass bone graft material supports and promotes the growth of bone in the fracture site. The healing process of a fracture field is unaffected by the nano bio glass bone transplant, which is a suitable material.
- In order to fill the fracture field, nano bio glass bone transplant offers high-quality newly formed bone.
- Mandibular angle fractures require special treatment plan design to overcome the unfavorable masticatory loads on it.

RECOMMENDATIONS

Further investigations are needed to improve the mechanical properties and the cost of the restorable systems.

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