Effects of hyaluronic acid on bone graft healing: An experimental study on sheep

Muhammed A. Mustafa[(1)]; Othman A. Omar[(2)]

Background and objectives: Alloplastic is one of known materials have been used to treat bony defects in oral and maxillofacial region, but the healing is much more slower than auto graft . So many interpositional graft materials like hyaluronic acid and bone morphogenetic protein have been introduced to such material to induce osteoinductivity of graft material. The aim was to evaluate the histological effect of Hyaluronic acid on bone graft substitute.

Materials and methods: An experimental study carried out on 3 domestic sheep 2-4 years old. They divided into two groups, first group (study group): bone graft substitute mixed with hyaluronic acid introduced to the created cavities in the basal bone of mandible. Second group (control group): bone graft substitute were introduced into created cavities in the basal bone of mandible and the defects in both groups were covered with collagen membrane. The samples then sacrificed in deferent period interval 2 weeks, 6 weeks and 12 weeks and the sample collected and sent for histological examination.

Results: All animals survived till the end of study. Both groups were characterized by new bone formation in defects however the bone formation in group 1 significantly preceded.

Conclusions: Hyaluronic acid induces the onset of new bone formation when mixed with alloplastic material.

Key words: Hyaluronic acid, bone graft, alloplastic material, guided bone regeneration.

[(1)]B.D.S., Master student at the College of Dentistry, Hawler Medical University.
[(2)]B.D.S., M.Sc., FIBMS. Assistant proffessor at the College of Dentistry, Hawler Medical University.

Introduction

Bone loss in oral and maxillofacial region result from trauma, tumor or cyst resection, infection, and also congenital defects like cleft palate.[1] Beside to aesthetical and psychological complain absence of this hard and soft tissue result in serious functional demand like mastication, speech, and also lead to compromise thermal and physical protection of important anatomical structures (i.e brain, vessels and nerves).[2]

Many treatment options have been introduced to repair bony defects in oral and maxillofacial region among surgeons . Autologus bone graft contain all of the basic elements that are necessary to induce effective tissue regeneration, provided cells, extracellular matrix and cytokines thus it consider as gold standard for bone replacement.[3] However taking autologous bone needs to be harvested from donor site with subsequent limitations related to cost, procedure time, and donor site morbidity.[4]

To overcome these confines, over the last three decades exogenous materials like allografts, xenografts and alloplasts, have been stated in clinically.[5] These materials act as scaffolds to maintain a space for cell migration from the periphery of the bed.[6] However when comparing to autografts, these biomaterials have some limitations. The presence of cellular popula-

tions, coordinate the release of growth factors, maintenance of a stable scaffold, and stimulate angiogenesis and are key for effective bone healing as they play an essential role on the healing process.[7] To promote those limitations bone substitutes combined with many biocompatible materials like hyaluronic acid and bone morphogenetic protein.[8]

Hyaluronic acid (HLA) is also called hyaluronate or hyaluronanis a high molecular weight polysaccharide that is also non-sulfated glycosaminoglycan that is distributed widely throughout connective tissue, synovial fluid and extracellular matrix of other tissues. HLA plays an important role in various biological cycles, including wound healing, chondrogenesis, immune response and cell migration.[8,9]

It has been recently reported that hyaluronic acid increases osteoblastic bone formation in vitro through increased mesenchymal cell differentiation and migration. Locally applied high molecular hyaluronic acid has also been shown to stimulate differentiation and migration of mesenchymal and muscular cells in vivo.[10]

It was also shown that high molecular weight hyaluronic acid inhibits osteoclast differentiation; cells specialized for bone resorption, by interacting with the Tool-like receptors (TLR4) present in them.[11] Hyaluronic acid based material is an interesting candidate for bone tissue engineering applications where a localized treatment is desirable as it is implicated in the induction of the mesenchymal stem cell migration by stimulating the over-expression of the cell surface cluster determinant 44 (CD44) receptor.[12]

Materials and methods

An experimental study carried out on three domestic sheep 40 to 60 Kg in weight aged 2-4 years after taking permission from ethical committee of Hawler medical university college of dentistry. All surgical procedures done at the veterinary theater (Qushtapa Veterinary Center-Erbil-Iraq).

Surgical procedures performed under intravenous sedation and local anesthesia the animals first took sedation using ketamine hydrochloride 0.3 mL/kg (KETALROM-50 ,S.C ROMVAC company, Ilfov,Romania) and xylazine (xyla; metaalweg 8,CG ventery, The Netherland), and 0.2 mg/kg local anesthesia consisting of lidocaine Hydrochloride 2% and Epinephrine1:100,000 (Lignospan, Louisville CO, 80027 by Novocol Pharmaceutical of Canada, Ontario, Canada) are administered locally in the surgical area (i.e to the basal bone of mandible). Then all surgical area were clipped, shaved, washed, and disinfected with povidone-iodine (Betadine). The lateral side of the basal bone of mandible exposed through long incision followed by blunt dissection and elevation of the skin and the facial layers. The bone graft recipient site were created using trephine bur 6 mm in diameter on slow speed handpiece with continuous cooling system six round hole prepared in each side of basal bone three superior hole filled with bone graft subistitute particles (30% hydroxyappetite plus 70% Beta tricalicum phosphate) (vial type OSTEON II , Dentium, south Korea) mixed with exogenous hyaluronic acid (HLA) ampoules (Suplasyn- Mylan- Germany), while the inferior three holes filled with same bone graft substitute alone. The holes then covered with resorbable collagen membrane (CYTOFLEX ®-USA). The area then closed in layers used vecryl 3:0 sutures.

The samples then sacrificed in three different period interval 2 weeks, 6weeks and 12 weeks the bone segment on which experiment has been done Perpendicular cross sections were taken and embedded in paraffin wax following fixation in formalin and formic acid decalcification. The sections were stained with hematoxylin eosin (HE) and examined under a light microscope. For histopathologic examination we depend on Emery's histopathological criteria Table 1.

Statistical analysis. Data were analyzed using the Statistical Package for Social Sciences (SPSS, version 22). Mann Whitney test (a non-parametric test) was used to compare the mean ranks of the Emery scale (ordinal scale) of the two study groups. A p value of ≤ 0.05 was considered statistically significant.

Table 1: Emery's histopathological criteria for bone healing (13)

Score	Tissue present
0	Empty cavity
1	Fibrous tissue only
2	More fibrous tissue than fibrocartilage
3	More Fibrocartilage than fibrous tissue
4	Fibrocartilage only
5	More fibrocartilage than bone
6	More bone than fibrocartilage
7	Bone only

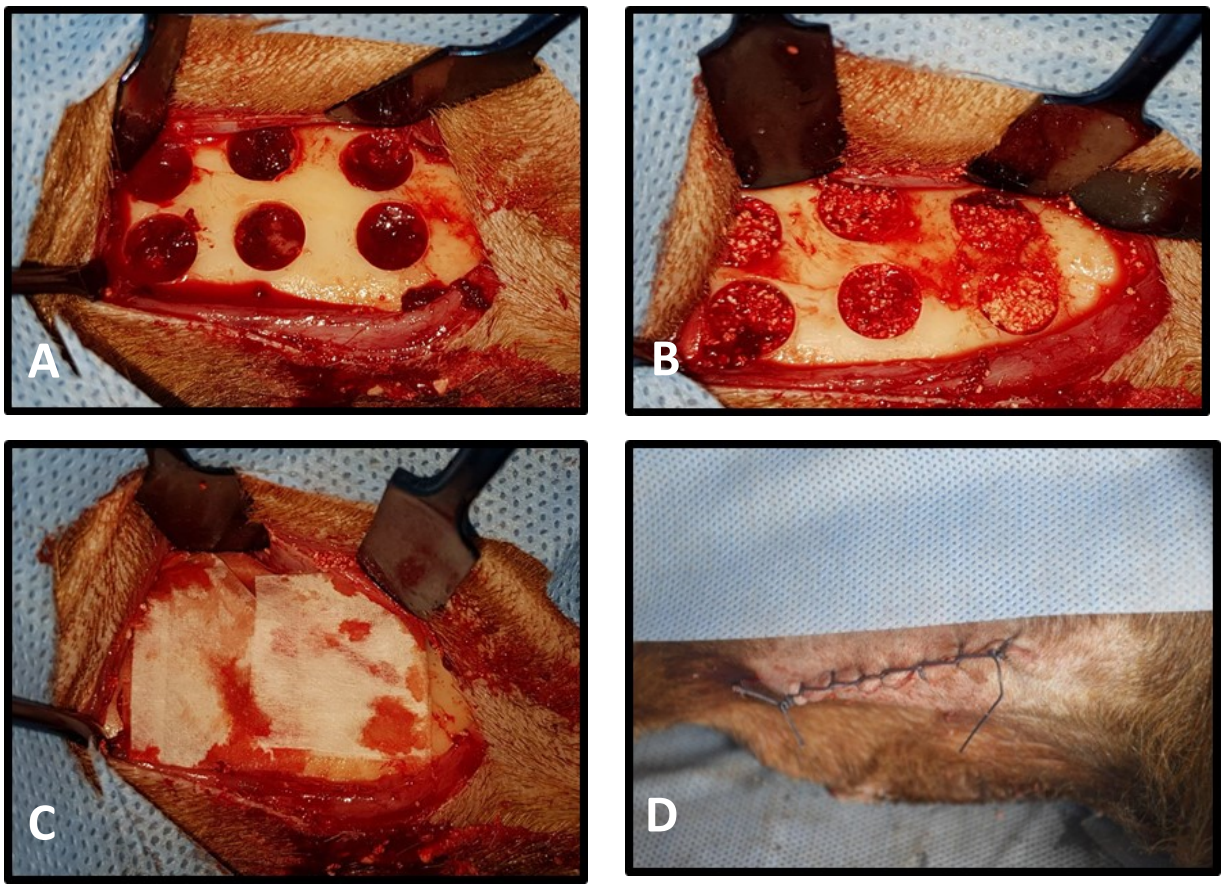

Figure 1: A- creation of six holes diameter of 6 mm. B-after filling with bone substitute alone (white arrow) and bone substitute + Hyaluronic acid (black arrow)C- placement of membrane D- the wound sutured primarily

Results

Eighteen bone substitute mixed with hyaluronic acid had been done placed inside eighteen created cavities in the basal bone of sheep mandible (G1), and another group (G2) was also composed of eighteen cavities , filled with a bone substitute without adding the hyaluronic acid.

After two weeks, six cavities from each group had been examined for bone healing (histopathological examination). In G1, the mean Emery scale was 2.33, the median was 2, and the range was 2 to 3. In G2, the mean was 1.33, with a median of 1, ranging from 1 to 2.

In group 1 there is evidence of fibrocartilage formation in all samples along with fibrous tissue whereas in group 2 no fibrocartilage have seen the areas filled with fibrous tissue and granulation tissue as shown in(figure 4 A and B).

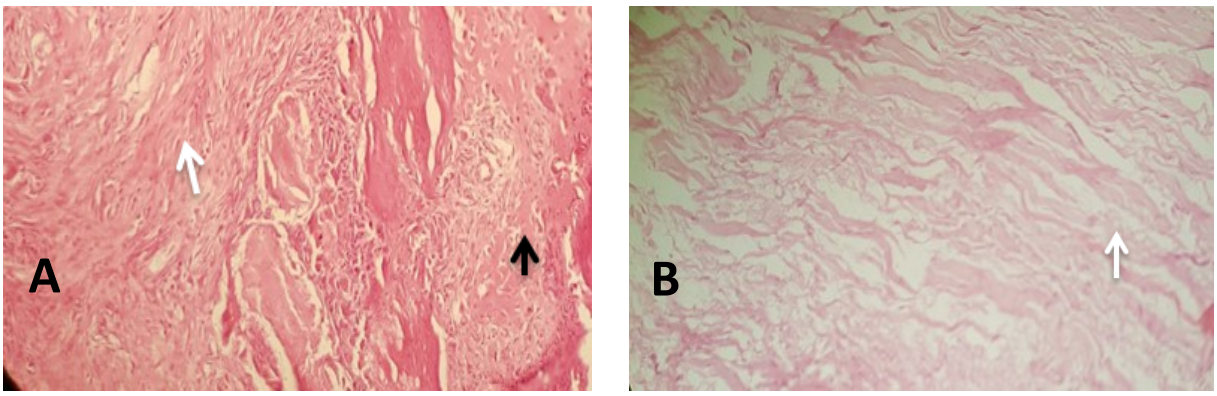

Figure 5: showing the histological picture of bone healing after two weeks. A in samples with bone substitute mixed with hyaluronic acid , B in sample treated with bone substitute alone. (H&E 400X) white arrow is fibrous tissue, black arrow is fibrocartilage.

After six weeks, another six cavities were examined histolopathologically from each group. In G1, the mean Emery scale increased to 4.67, the median was 5, and the range was 4 to 5. In G2, the mean increased to 3.33, the median was 3, and the range was 3 to 4.

The fibrocartilagenous change obviously seen beside of bone formation in four samples in G1, whereas in G2 there were still fibrous tissue remained along with formation of fibrocartilage however, fibrous tissue were absent in two cavities (figure 6 A and B).

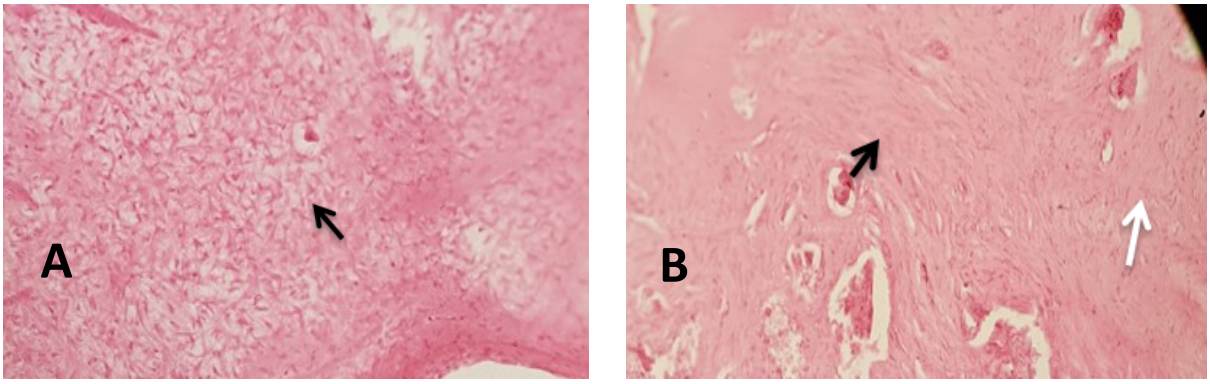

Figure 6: showing the histological picture of bone healing after 6 weeks. A in samples with bone substitute mixed with hyaluronic acid , B in sample treated with bone substitute only. (H&E 400X) white arrow is fibrous tissue, black arrow is fibrocartilage

After 12 weeks, six cavities were also examined from each group. In G1 the mean of Emery scale increased to 6.5, the median was also 6.5, and the range was 6 to 7. In G2, the mean was 5.17, the median was 5, and the range was 5 to 6 (Table 1).
There was complete bone formation in hyaluronic group G1 while other group there is still area filled with fibrocartilage.

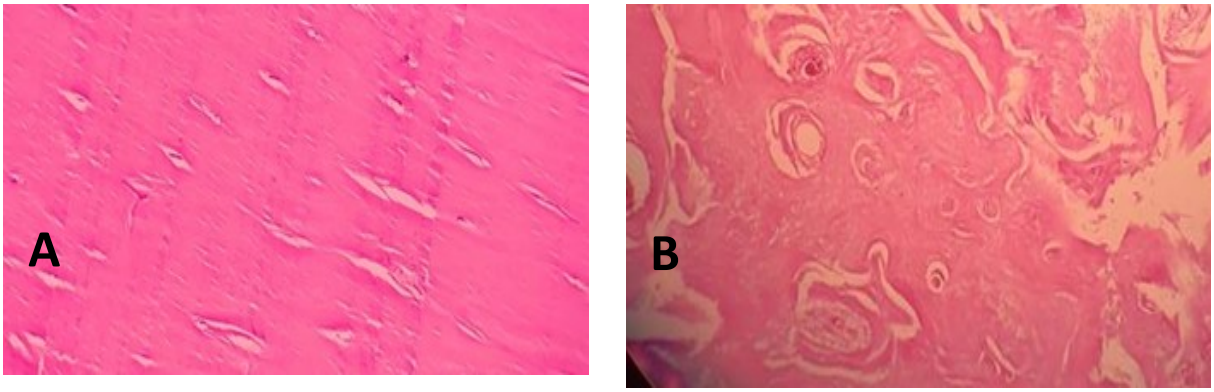

Figure 7: showing the histological picture of bone healing after 12 weeks. A in samples with bone substitute mixed with hyaluronic acid , B in sample treated with bone substitute alone. (H&E 400X)

Table 2: Descriptive statistics of the 'Emery classification scale' of histo-pathological bone healing of the two study groups.

	Hyaluronic acid group			Without acid group		
	WK 2	**WK 6**	**WK 12**	**WK 2**	**WK 6**	**WK 12**
N	6	6	6	6	6	6
Mean	2.33	4.67	6.50	1.33	3.33	5.17
SD	0.516	0.516	0.548	0.516	0.516	0.408
Median	2.00	5.00	6.50	1.00	3.00	5.00
Minimum	2	4	6	1	3	5
Maximum	3	5	7	2	4	6

Table 3:Comparison of the mean ranks of the Emery scale of the two study groups by weeks.

	The study groups		
	Hyaluronic acid	**No acid**	**p***
	Emery mean rank	**Emery mean rank**	
Week 2	8.83	4.17	0.014
Week 6	9.17	3.83	0.007
Week 12	9.25	3.75	0.005

*By Mann Whitney test.

Discussion

The treatment of craniomaxillofacial defects is one of the greatest challenges in bone healing.[8] Osteon II is an alloplastic materials composed of 30% hydroxyapatite and 70 % beta-tricalcium phosphate. generally it has osteoconductive properties its chemical and structural characters are similar to those of human bone.[14] However, modern tissue engineering technology makes it is possible to design new scaffolds and tissue grafts to improve osteogenic, osteoinductive, and osteoconductive effects and to mitigate certain disadvantages of these grafts.[15]

There are three phases in normal bone healing including inflammation, proliferation and migration of mesenchymal steam cells and bone remodeling subsequently.[16] All these phases are present in our experiment which was done on damaged bone and filled with bone graft subistitute and hyaluronic acid.

The terminology of bone grafting is complicated by the various histologic types of bone grafts. It can be classified based on the site were it taken from autograft is a graft that transferred from one site to another within the same individual. Isograft is a transferred tissue from one twin is into an identical (monozygotic) twin. An allograft is the tissue transferred between two genetically different individuals of the same species. xenograft (heterogen) is the tissue from one species implanted into a member of a different species. An alloplastic graft is a biological implant which is synthetically produced.[17] Ideally, graft subtitutes should provide three elements:[13]

An osteoconductive matrix: In which bone substitute act as scaffolding conducive to make a space for bone to grow.

Osteoinductive factors: any chemical agent that induce the various stages of bone regeneration and repair.

Osteogenic cells: which have the possible to differentiate and simplify the various stages of bone revival and structural integrity.

It is important to fill any maxillofacial bony defect as soon as possible prior to any reconstruction. Therefore, in our study we evaluated the effect of hyaluronic acid in conjunction with bone substitute and evaluated the result appeared that the combination of alloplast plus hyaluronic acid resulted in statistically significantly greater bone fill than the alloplast alone.

These results support our hypothesis that hyaluronate promotes new bone formation, most probably by increasing the osteoinductive effect of the alloplast. In this experimental study performed on sheep mandible , we can point the osteoinductivity of hyaluronic acid to induced growth factors, such as bone morphogenetic proteins.[8] Bone morphogenetic proteins are known to play important roles in the migration of progenitor cells, proliferation, and differentiation of mesenchymal cells to osteogenic cells, vascular invasion, and bone remodeling.[18]

Conclusion

Our study suggest that there is bone formation in both group after three months however the bone formation is significantly more in group that treated in mixture with hyaluronic acid.

Conflicts of interest

The author reported no conflict of interests.

References

1. Pagni G, Kaigler D, Rasperini G, Avila-Ortiz G, Bartel R, Giannobile W. Bone Repair Cells for Craniofacial Regeneration. Adv Drug Deliv Rev. 2012 Sep;64(12):1310–9. https://doi.org/10.1016/j.addr.2012.03.005
2. Davis RE, Telischi FF. Traumatic facial nerve injuries: review of diagnosis and treatment. J Craniomaxillofac Trauma. 1995;1(3):30–41.
3. Pape HC, Evans A, Kobbe P. Autologous bone graft: properties and techniques. J Orthop Trauma. 2010 Mar;24 Suppl 1:S36-40. https://doi.org/10.1097/BOT.0b013e3181cec4a1
4. Zouhary KJ. Bone graft harvesting from distant sites: concepts and techniques. Oral Maxillofac Surg Clin North Am. 2010 Aug;22(3):301–16, v.
5. De Long WG, Einhorn TA, Koval K, McKee M, Smith W, Sanders R, et al. Bone grafts and bone graft substitutes in orthopaedic trauma surgery. A critical analysis. J Bone Joint Surg Am. 2007 Mar;89(3):649–58. https://doi.org/10.2106/JBJS.F.00465
6. Finkemeier CG. Bone-grafting and bone-graft substitutes. J Bone Joint Surg Am. 2002 Mar;84-A (3):454–64. https://doi.org/10.1111/j.1399-3089.2012.00694.x
7. Taba M, Jin Q, Sugai JV, Giannobile WV. Current

concepts in periodontal bioengineering. Orthod Craniofac Res. 2005 Nov;8(4):292–302. https://doi.org/10.1111/j.1601-6343.2005.00352.x
8. Arpağ OF, Damlar I, Altan A, Tatli U, Günay A, Arpağ OF, et al. To what extent does hyaluronic acid affect healing of xenografts? A histomorphometric study in a rabbit model. Journal of Applied Oral Science [Internet]. 2018 [cited 2018 Oct 31];26. https://doi.org/10.1590/1678-7757-2017-00049.
9. Bertl K, Bruckmann C, Isberg P-E, Klinge B, Gotfredsen K, Stavropoulos A. Hyaluronan in non-surgical and surgical periodontal therapy: a systematic review. J Clin Periodontol. 2015 Mar;42 (3):236–46. https://doi.org/10.1111/jcpe.12371
10. Zhao N, Wang X, Qin L, Guo Z, Li D. Effect of molecular weight and concentration of hyaluronan on cell proliferation and osteogenic differentiation in vitro. Biochem Biophys Res Commun. 2015 Sep 25;465(3):569–74. https://doi.org/10.1016/j.bbrc.2015.08.061
11. Chang E-J, Kim HJ, Ha J, Kim HJ, Ryu J, Park K-H, et al. Hyaluronan inhibits osteoclast differentiation via Toll-like receptor 4. Journal of Cell Science. 2007 Jan 1;120(1):166–76. https://doi.org/10.1242/jcs.03310
12. Zhu H, Mitsuhashi N, Klein A, Barsky LW, Weinberg K, Barr ML, et al. The role of the hyaluronan receptor CD44 in mesenchymal stem cell migration in the extracellular matrix. Stem Cells. 2006 Apr;24(4):928–35. https://doi.org/10.1634/stemcells.2005-0186.
13. Aslan M, Simsek G, Dayi E. The effect of hyaluronic acid-supplemented bone graft in bone healing: experimental study in rabbits. J Biomater Appl. 2006 Jan;20(3):209–20. https://doi.org/10.1177/0885328206051047
14. Omar Naser Ihghaf N, Abdel- Monem Tawfik M, Elhawary Y, Ahmed Mansour N. OSTEON II VERSUS BIOGEN IN HEALING OF JAW BONE DEFECTS. Egyptian dental journal. 2015 Jul 1;61:4045:4053.
15. Oryan A, Alidadi S, Moshiri A, Maffulli N. Bone regenerative medicine: classic options, novel strategies, and future directions. J Orthop Surg Res. 2014 Mar 17;9(1):18. https://doi.org/10.1186/1749-799X-9-18
16. Mescher A. Junqueira's Basic Histology. 12th Revised edition edition. New York: McGraw-Hill Medical; 2009. 467 p.
17. Fernández RF, Bucchi C, Navarro P, Beltrán V, Borie E. Bone grafts utilized in dentistry: an analysis of patients' preferences. BMC Med Ethics [Internet]. 2015 Oct 20 [cited 2018 Sep 23];16. https://doi.org/10.1186/s12910-015-0044-6
18. Long B, Dan L, Jian L, Yunyu H, Shu H, Zhi Y. Evaluation of a novel reconstituted bone xenograft using processed bovine cancellous bone in combination with purified bovine bone morphogenetic protein. Xenotransplantation. 2012 Apr;19(2):122–32.