

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

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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. This study aimed to evaluate the histological effect of Hyaluronic acid on bone graft substitute.

Patients 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.

Conclusion: hyaluronic acid induces the onset of of Oral and Maxillofacial department.

Keywords: hyaluronic acid, Bone graft, Alloplastic material, guided bone regeneration.

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Introduction

Bone loss in oral and maxillofacial region may 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 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 Many treatment options have been introduced to repair bony defects in oral and maxillofacial 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 populations, 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

substitutes combined with many biocompatible materials like hyaluronic acid and bone morphogenetic protein.⁸

Hyaluronic acid (HLA) is also called hyaluronate or hyaluronan is 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 Toll-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 Epinephrine 1:100,000 (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 substitute particles (30% hydroxyapatite plus 70% Beta tricalcium 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, 6 weeks 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.

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

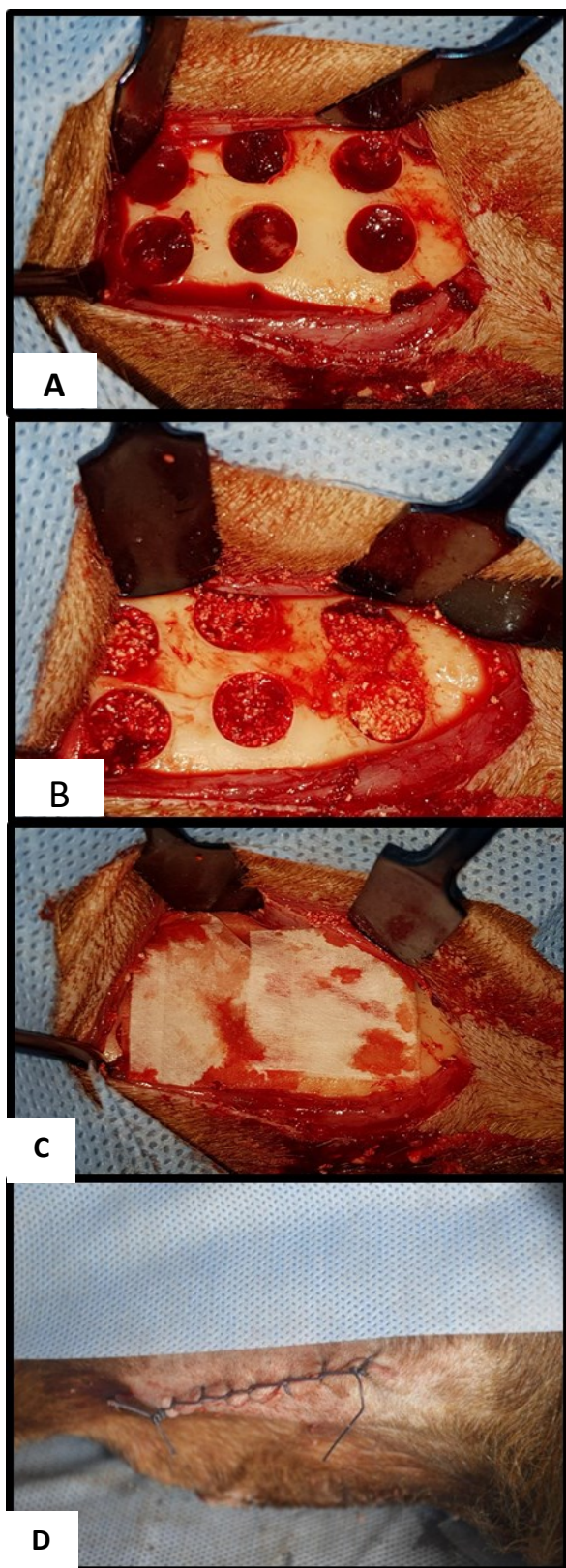


Figure 1: A- creation of six holes diameter of 6 mm. B-after filling with bone substitute alone (white arrow) and bone substitute + Hyaluronic acid (black arrow)C- placement of membrane D- the wound sutured primarily.

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.

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).

After six weeks, another six cavities were examined histopathologically 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).

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.

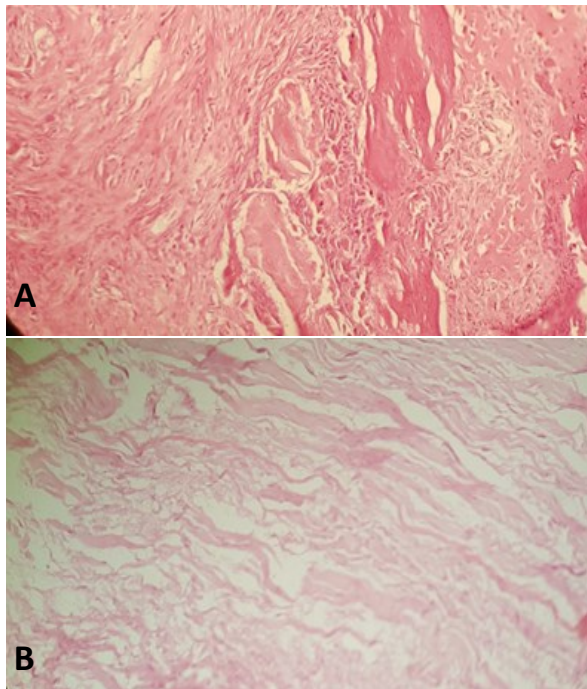


Figure 5: showing the histological picture of bone healing after two weeks. A in samples with bone substitute mixed with hyaluronic acid , B in sample treated with bone substitute alone. (H&E 400X) white arrow is fibrous tissue.

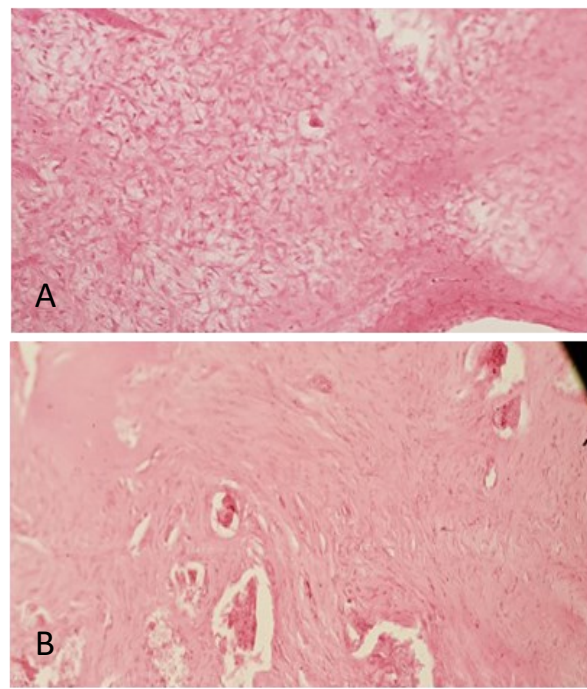


Figure 6: showing the histological picture of bone healing after 6 weeks. A in samples with bone substitute mixed with hyaluronic acid , B in sample treated with bone substitute only. (H&E 400X) white arrow is fibrous tissue.

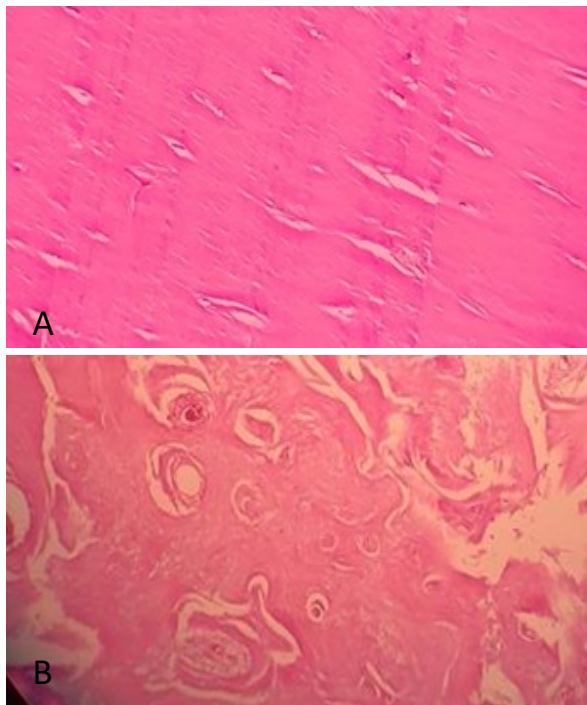


Figure 7: showing the histological picture of bone healing after 12 weeks. A in samples with bone substitute mixed with hyaluronic acid , B in sample treated with bone substitute alone. (H&E 400X).

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	.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		p*
	Hyaluronic acid	No acid	
	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 stem 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 substitute 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 substitutes should provide three elements¹³:

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 authors reported no conflicts of interests.

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