Histological and histomorphometric studies of the effects of hyaluronic acid on osseointegration of titanium implant in rabbits

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ABSTRACT

Background: One of the unique prosthesis for tooth or teeth replacement is the dental implant. Our attempt is using a biomaterial system that is easily obtained and applicable and has the ability to provoke osteoinductive growth factor to enhance bone formation at the site of application. One of these natural polymers is hyaluronic acid.

Material and methods: Sixty machined surface implants from commercially pure titanium rod inserted in thirty NewZealand rabbits. Two implants placed in both tibia of each rabbit. The animals scarified at 1, 2 and 4 weeks after implantation (10 rabbits for each interval). For all of animals the right tibia's implant was control (uncoated) and the left one was experimental (coated with 0.1ml Hyaluronic acid gel). All sections have been stained with Haematoxylin and Eosin then they were histologically examined and assessed for histomorphometric analysis for counting of bone cells (osteoblast, osteocyte and osteoclast), cortical bone thickness, trabecular width, thread width and marrow space star volume (V*).

Results: Histological findings for hyaluronic acid- coated titanium implant revealed an earlier bone formation, mineralization and maturation than that in control groups. Histomorphometric analysis for all bone parameters that examined in this study, showed highly significant difference between control and experimental groups in all healing intervals.

Conclusion: Commercially pure titanium endosseous implants coated with hyaluronic acid may be osteocoductive thus accelerating healing process and enhancing osseointegration.

Key words: bone, implant, hyaluronic acid, osseointegration. **(J Bagh Coll Dentistry 2018; 30(2):10-16)**

INTRODUCTION

Dental implant or fixture is a surgical component that interfaces with the bone of the jaw to support a dental prosthesis [(1)]. The basis for modern implants is a biologic process called osseointegration where materials, such as titanium, form an intimate bond to bone [(2)].

Osseointegrated implant is a type of implant defined as "an endosteal implant containing pores into which osteoblasts and supporting connective tissue can migrate. Applied to oral implantology, this refers to bone grown right up to the implant surface without interposed soft tissue layer [(1) (3)].

Various techniques of surface treatments have been introduced and applied to enhance surface properties of titanium implants, as a result supported osseointegration through encouraged bone formation and better implant stability [(4)].

Hyaluronan (hyaluronic acid) is considerd to be one of the fundamental constituent of connective tissue and bone marrow extracellular matrix. It mediates to chemotaxis, proliferation and successive differentiation of mesenchymal cells so it plays an essential function in regeneration and repaire of tissue [(5)].Due to its osteogenic induction ability, biocompatibility and non-immunogenic nature and it believed to have angiogenic properties [(6)] HA coating process increases the hydrophilic nature of the implant surface such that the growth factors and proteins necessary for osseointegration are more readily attracted to the implant surface and increase the rate at which the bone heals [(7)].

MATERIALS AND METHODS

Sixty machined surface implants from commercially pure titanium rod inserted in 30 adult male New Zealand white rabbits aged from 10 - 12 months and their weights were between 1.5 - 2 kgs. Two implants placed in both tibia of each rabbit, one in right tibia as control and another one in the left tibia as an experimental. The animals scarified at 1, 2 and 4 weeks after implantation (10 rabbits for each interval).

The implants categorized as control group (30 uncoated implants), 10 implants for each healing intervals and experimental group (30 hyaluronic acid coated implants), 10 implants for each healing intervals.

The sterilization implants were placed in the hole of 5 mm in prepared in both rabbit's tibiaes. The insertion of the uncoated one was directly done in the right tibia, while the insertion of the coated implants was performed in the left tibia after the application 0.1ml hyaluronic acid gel inside the threaded part of implants.

After the rabbits were sacrificed at the end of recommended periods. The right and left tibias were dissected and the soft tissue was removed to

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expose the entire bone to be cut at 5 mm away of both implants sides to make implant contained bone blocks.

The specimens were fixed in 10% formalin for 48h, decalcified with solution of formic acid, then bone tissue dehydrated with alcohol and embedded in paraffin. Sections of 5µm were prepared in the usual fashion, and stained with hematoxylin and eosin. Histological examination was performed using light microscope. Histomorphometric assessment of bone cells (osteoblast, osteocyte and osteoclast), cortical bone thickness, trabecular width, thread width and marrow space star volume (V*).was done

HISTOLOGICAL FINDINGS:

One week duration

A-Control group

The histological finding showed deposition of fibrous connective tissue with osteoid tissue in some area **(Figure 1).** The thread area filled with osteoblasts, inflammatory cells, fibroblasts and progenitor cells **(Figure 2).**

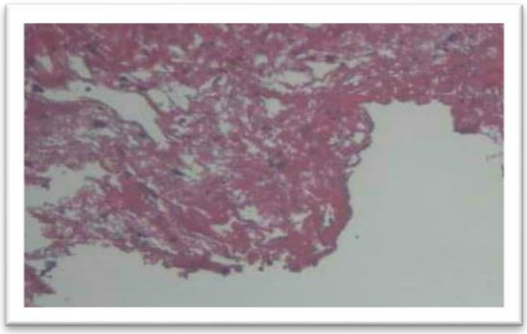

Figure 1: Histological view of 1 week duration control group shows thread area filled with fibrous C.T. and osteiod tissue. H&E X10.

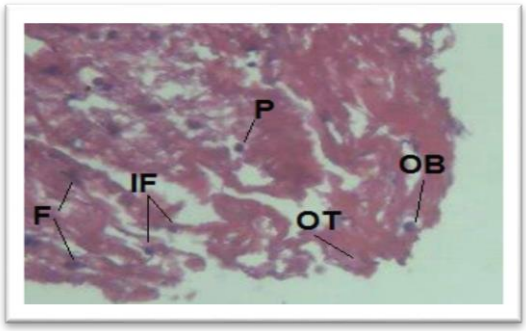

Figure 2: Magnifying view shows progenitor cells (p), inflammatory cells(IF), fibroblast (F), osteoblast(OB) and osteoid tissue(OT),H&E. X20.

B-Experimental group:

The histological findings revealed woven bone in the thread area which was followed the implant shape **(Figure 3)**. In higher magnification the thread area filled with thin bone trabecluae with osteoblasts arranged in a single raw at the edges of these trabeculae, osteocytes were occupying their large lacunae **(Figure 4 and 5).**

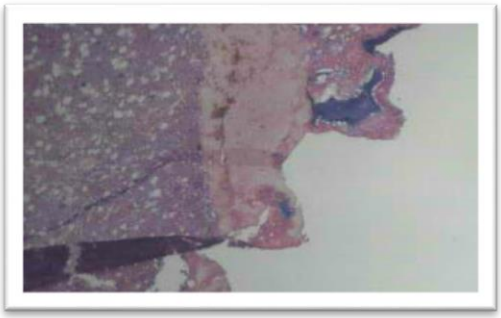

Figure 3: View of one week experimental group reveals woven bone in the thread area which was followed the screw shape and hyalinization of blood vessels H&E X4

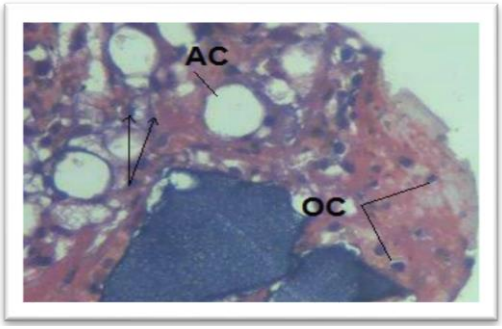

Figure 4: Magnifying view shows thread with osteocytes (OC), adipose cells(AC), hyalinization of blood vessels and inflammatory cells (arrows) .H&E X20

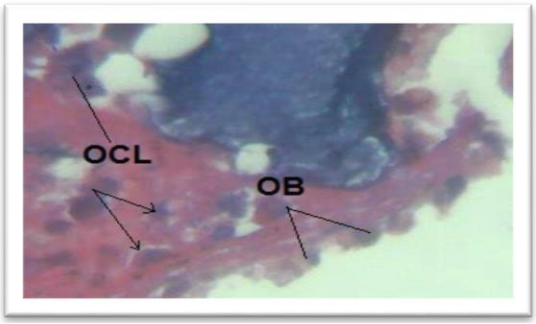

Figure 5: View of one week experimental group shows bone trabecullae filled with osteocytes (arrows) and lined by osteoblasts, osteoclasts (OCL).H&E X40.

At 2 weeks duration

A-Control group:

The histological view showed woven bone with few thin bone trabeculae filled with preosteocytes and osteocytes **(Figure 6, 7)**.

B- Experimental group:

The histological view of 2weeks experimental group shows new bone trabeculae which

demarcated from basal bone by reversal line. These bone trabeculae filled with osteocytes and surrounded by osteoblast and osteoclasts **(Figure 8, 9).**

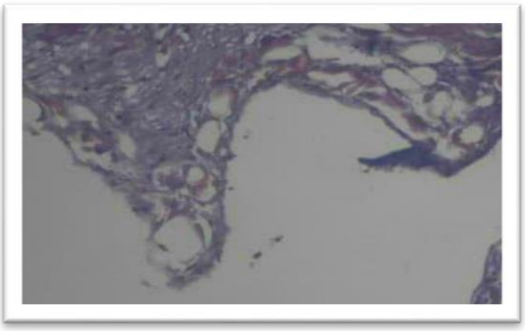

Figure 6: Histological view of 2week control group shows woven bone and thin bone trabecullae filled thread area .H&E X10

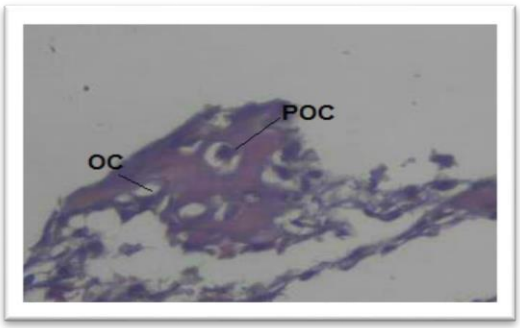

Figure 7: Magnifying view shows new bone trabecullae filled with large size osteocyte(OC) and preosteocytes(POC).H&E X10.

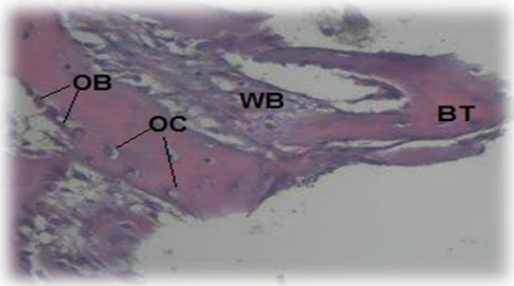

Figure 8: View of 2weeks experimental group shows woven bone(WB), new bone trabecullae (BT) filled thread area with osteoblasts(OB) and osteocytes(OC). H&E X20.

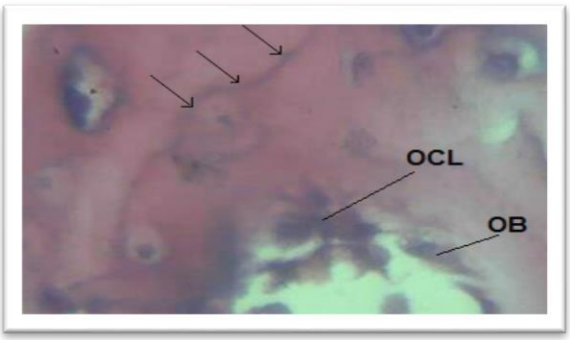

Figure 9: Magnifying view shows reversal line (arrows) which separate between basal and new bone with osteoblasts(OB) and osteoclasts (OCL) . H&E X100

At 4weeks duration

A-Control group:

The histological view shows formation of dense bone trabecullae filled with large size osteocytes and surrounded by active osteoblasts **(Figure 10).** Also presence of osteoclasts and reversal line in higher magnification this indicate continuous bone remodeling **(Figure 11)**.

B-Experimental group

The histological view reveals well established mature bone rimmed by osteoblast and filled with osteocytes occupied their small lacunae in thread area **(Figure 12).** In higher magnification the mature bone characterized by presence of Haversion lamellae **(Figure 13)**.

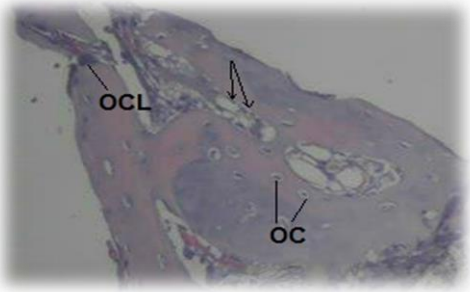

Figure 10: View of 4 weeks control group shows osteoblasts (arrows), osteoclasts(OCL), and osteocytes(OC) in thread area. H&E X20

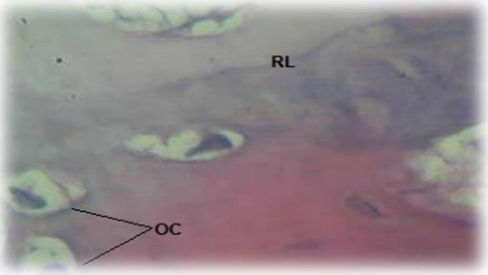

Figure 11: Magnifying view of 4 weeks control group shows osteocytes (OC) and reversal line (RL). H&E X100

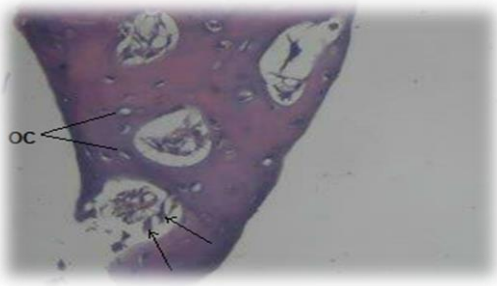

Figure 12: Histological view of 4 weeks experimental group shows mature bone rimmed by osteoblasts (arrows) and osteocytes(OC) in thread area. H&E X20.

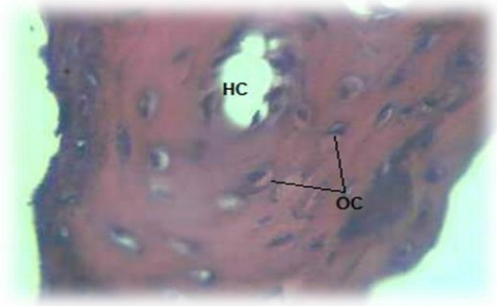

Figure 13: Magnifying view of 4 weeks experimental group shows Haversian lamellae with osteocytes (OC) that arranged in a circle around Haversian canal(HC). H&E X40.

Histomorphoetric analysis of studied groups for bone architecture parameters:

The descriptive statistics of bone architecture parameters for control and experimental groups in all healing intervals are shown in **(Table 1)**. For both control and experimental groups, the mean values of cortical bone thickness, trabecular width and thread width are increase with proceeded time. The mean values for HA-coated group are higher than those in control group in all recovery periods. With advancing healing time marrow space star volume mean values are decreased in both groups, with noticeable reduction in HA coated group in comparison to control ones**.**

The osteoblasts and osteocytes number mean values for both experimental and control groups increased with time, with increase in the mean values for HA coated group than that for control one in 1 and 2 week intervals. Regarding the osteoclasts number, the mean values were in week 2 for both groups with less osteoclasts number in HA treated group than that of control one at the same interval**.**

Statistically all histomorphometric variables, showed a highly significant difference between the control and experimental groups in all healing intervals except osteoblasts and osteocytes number that showed non-significant difference in 2 and 4weeks , and non-significance differences were noted with osteoclasts in all healing intervals **(Table 2).**

Table (1) Descriptive statistics for bone architecture parameters for all groups.

Variables	Duration	Control group						Experimental group					
		N	Mean	S.D.	S.E.	Min.	Max.	N	Mean	S.D.	S.E.	Min.	Max.
Cortical bone thickness	1 weeks	5	0.87	0.04	0.02	0.79	0.97	5	2.02	0.04	0.02	1.9	2.1
	2 weeks	5	1.84	0.03	0.01	1.79	1.87	5	2.8	0.06	0.02	2.7	2.9
	4 weeks	5	2.07	0.13	0.05	1.82	2.21	5	3.3	0.13	0.04	3.1	3.5
Trabecular width	1 weeks	5	0.13	0.01	0.003	0.12	0.14	5	0.46	0.01	0.003	0.45	0.48
	2 weeks	5	0.21	0.004	0.001	0.21	0.22	5	0.6	0.04	0.02	0.56	0.7
	4 weeks	5	0.34	0.01	0.003	0.33	0.35	5	0.75	0.02	0.01	0.69	0.78
Thread width	1 weeks	5	0.24	0.017	0.006	0.22	0.26	5	0.63	0.03	0.01	0.6	0.67
	2 weeks	5	0.32	0.02	0.006	0.3	0.35	5	0.73	0.02	0.008	0.71	0.79
	4 weeks	5	0.33	0.04	0.014	0.28	0.39	5	0.75	0.32	0.01	0.72	0.79
Marrow space star volume	1 weeks	5	0.055	0.001	0.001	0.05	0.06	5	0.009	0.003	0.001	0.001	0.01
	2 weeks	5	0.052	0.003	0.001	0.052	0.06	5	0.007	0.001	0.001	0.004	0.009
	4 weeks	5	0.035	0.001	0.001	0.032	0.04	5	0.001	0.001	0.0001	0.0008	0.001
Osteoblasts No.	1 weeks	5	4.9	2.6	0.9	2	10	5	7.25	1.38	0.49	6	10
	2 weeks	5	11.2	4.6	1.6	5	19	5	15.1	5.27	1.86	10	25
	4 weeks	5	16.3	9.5	3.3	9	32	5	13.88	8.32	2.94	8	31
Osteocytes No.	1 weeks	5	0	0	0	0	0	5	2	1.07	0.37	1	4
	2 weeks	5	7.2	4	1.4	3	13	5	9	4.27	1.51	3	15
	4 weeks	5	10.7	4.4	1.5	3	16	5	10.5	4.59	1.62	3	17
Osteoclasts No.	1 weeks	5	0.25	0.46	0.16	0	1	5	0.38	0.51	0.18	0	1
	2 weeks	5	1.38	1.5	0.53	0	4	5	1	1.41	0.5	0	4
	4 weeks	5	0.88	0.83	0.29	0	2	5	0.88	0.64	0.22	0	2

Table (2) Groups' comparison for all histomorphometric variables in each duration

Variables	Duration	Groups' Comparisons d.f.** = 14	
		t-test	p-value*
Cortical bone thickness	1 week	49.246	0.000 (HS)
	2 weeks	43.472	0.000 (HS)
	4 weeks	18.407	0.000 (HS)
Trabecular width	1 week	73.462	0.001 (HS)
	2 weeks	25.602	0.000 (HS)
	4 weeks	44.635	0.001 (HS)
Thread width	1 week	32.694	0.000 (HS)
	2 weeks	38.75	0.000 (HS)
	4 weeks	22.896	0.000 (HS)
Marrow space star volume	1 week	-31.679	0.000 (HS)
	2 weeks	-38.922	0.000 (HS)
	4 weeks	-64.134	0.000 (HS)
Osteoblasts No.	1 week	2.287	0.038 (S)
	2 weeks	1.563	0.14 (NS)
	4 weeks	-0.56	0.584 (NS)
Osteocytes No.	1 week	5.292	0.000 (HS)
	2 weeks	0.839	0.415 (NS)
	4 weeks	-0.111	0.913 (NS)
Osteoclasts No.	1 week	0.509	0.619 (NS)
	2 weeks	-0.513	0.616 (NS)
	4 weeks	0.0	1.0 (NS)

***** HS: highly significant, S: significant, NS: non-significant, **d.f. = degree of freedom

DISCUSSION:

The goal of present study was to evaluate the effects of hyaluronic acid on bone-implant interface. Hyaluronan is considerd to be one of the fundamental constituent of connective tissue and bone marrow extracellular matrix. it plays an essential function in regeneration and repair of tissue [5]. Due to its osteogenic induction ability, biocompatibility and non-immunogenic nature led to its use in a number of clinical applications, such as fabricating and/or coating an implant or other structure to be inserted into bone or osseous tissue and to facilitate the healing and regeneration of bone [7]. For both control and experimental groups the histological observations revealed that all sections run in a good healing path with variance in bone deposition and remodeling rate for each healing intervals. After one week of implantation in control animals, the sections showed clear blood clot replacement by granulation tissue containing abundant collagen fibers, considerable number of fibroblasts and osteoblasts with starting of osteoid tissue formation. While in HA treated implant the granulation tissue were already started to be replaced by new bone by osteoblast differentiation. This findings agree with **Mendes *et al.*, 2010** [9] which confirmed the healing of upper first molar extraction socket in rat treated with HA was fast and more organized bone matrix formation after one week due to deposition of bone trabeculae. Also in agreement with **Baisse *et al.*, 2004** [10] who mentioned that after I week of rabbit tooth extraction and HA application in socket promoted and facilitated blood clot substitution by granulation tissue promotes alveolar bone consolidation with an evidence of filling at the apex with fine, nascent bridges. After two weeks interval the histological sections of control group showed delicate bone trabeculae with newly formed woven bone. While HA treated group showed more and thicker bone trabeculae than that of control one. These findings were in agreement with **Sanz *et al.*** [11] who noticed that after two weeks of HA treated sockets after rat tooth extraction, more

bone trabeculae was formed when compared with that in control one.

At four weeks interval the histological sections showed immature bone in control group while well developed, mature (lamellar) bone formation in experimental ones. This result in agreement with previous study done by **Elkarargy** [12] who found that the mixing of hyaluronic acid with hydroxyapatite/beta tricalcium phosphate induced more bone formation efficiently in comparison with using of hydroxyapatite /beta tricalcium phosphate alone. The equality of means and variance of all parameters tested for micro architecture records between control and experimental groups illustrated a high value in experimental groups than those of control groups, this result can be explained on a fact of early induction of the progenitor cells to be differentiated into osteoblasts and enhancement of osteoid tissue formation**.** Entrapment of osteoblast in their matrix led to osteocyts formation. More and faster bone matrix construction resulted from more osteoblast formation and consequently more osteocytes**.** The results also showed that there was a significant difference in bone architecture parameters in different intervals time. Increase in trabecular width, cortical width, thread width, and trabecular number in 2and 4 weeks in comparison to 1 weeks which could be attributed to time spending for bone deposition and maturation, while the decrease in bone marrow star volume with the time could be due to the fact that faster building of bone matrix, bone trabeculae width will be more wider and there will be less bone star volume (V). These finding were agree with the study done by **Depprich *et al.*** [13] who found that the histomorphometric analysis revealed an enhanced bone-to implant contact for every healing period. The results also showed that there was a significant difference in bone architecture parameters in different intervals time. Increase in trabeculae width, and cortical width resulted in decrease of (V), while the decrease in number of osteoblasts and increase in number of osteocytes with increase time could be explained on a fact that any new tissue formation needs for more osteoblasts, when the formation of the bone settled and reached to its final measurement no more osteoblasts are required except for preservation of the biological activity. This results agree with **Al-Molla *et al.*** [14] and **Al-Molla** [15] who found that the number of osteoblasts decrease by the time while osteocytes number increased between the groups in second week & 4 week intervals.

CONCLUSION

Results obtained in this study have shown that hyaluronic acid is osteoconductive material that enhance and accelerates osseointegration around titanium implant by stimulating the osteogenic mesenchymal tissue and osteoblast differentiation and then early apposition of osteoid tissue.

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