Histological and mechanical evaluation of the osseointegration of titanium implants by the modifications of thread design and/or coating with flaxseed (An experimental study on rabbits)

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ABSTRACT

Background: Dental implant surface technologies have been evolving rapidly to enhance a more rapid bone formation on their surface and improve implant therapy.Implant threads should be designed to increase surface contact areathat induced better stability. In addition, implant surface coating with Flaxseed was used to enhance bone formation at the bone-implant interface.

Materials and methods: Ninety-six commercially pure titanium (CpTi) screws were implanted in rabbits' tibiae and divided into three groups as dual-threaded group, flaxseed-coated group and control group. All groups were evaluated mechanically, histologically and radiographically after each healing periods (2, 4, 6 and 8) weeks and the resulting data were statistically analyzed using ANOVA and t-test at 0.05 significance level.

Results: Dual threaded implant recorded the highest value in removal torque testand it showed mature bone at 8 weeks period. Coated implant shows enhancement of osteoblast and it is the only modified implant that illustrates proliferating cartilage zone that later on degenerated and replaced by bone.

Conclusion: Each modified implants shows different benefits whether a modification of the implant surface mechanically (dual-threaded) or by coating the implants with Flaxseed.

Keywords: Bone formation, titanium implant, thread design, flaxseed. (J Bagh Coll Dentistry 2015; 27(2):72-78).

INTRODUCTION

Implants could be considered predictable tools for replacing missing teeth or teeth that are irrational to treat [(1)]. Dental Implant is defined as a "prosthetic device of alloplastic material(s) implanted into theoral tissues beneath the mucosal and/or periosteal layer, and/or within the bone to provide retention and support for a fixed or removable prosthesis" [(2)]. Today, implant success is evaluated from the esthetic and mechanical perspectives. Both depend on the degree and integrity of the bond created between the implant and the surrounding bone. Osseointegration actually refers to a structural and functional fusion of the implant surface with the surrounding bone. Many factors affect osseointegrationsuch as surgical technique, host bed, implant design, implant surface, material biocompatibility and loading conditions [(3)].

Implant design refers to the three-dimensional structure of the implant, with all the elements and characteristics that compose it.

Implant design features are one of the most fundamental elements that have an effect on implant primary stability and implant ability to sustain loading during or after osseointegration.

Hence, Implant threads should be designed to maximize the delivery of optimal favorable stresses while minimizing the amount of extreme adverse stresses to the bone implant interface. In addition, implant threads should allow for better stability and more implant surface contact area [(4)].

The thread lead and pitch are important thread characteristics that affect bone-implant contact, stress distribution and primary stability. *Thread pitch* refers to the distance from the center of the thread to the center of the next thread, measured parallel to the axis of a screw [(5)].Pitch differs from *lead*, which is the distance from the center of the thread to the center of the same thread after one turn or, more accurately, the distance that a screw would advance in the axial direction if turned one complete revolution.In a single-threaded screw, lead is equal to pitch; however in a double threaded screw; lead is double the pitch (Figure 1). An implant with double threads would insert twice as fast the single threaded [(4)]. Studies found that implants with more threads (lower pitch) had a higher percentage of bone-implant contact. The pitch is considered to have a significant effect among implant design variables, because of its effect on surface area [(6)].

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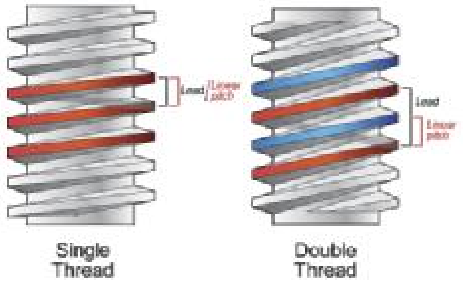

Figure 1: Single threaded and dual threaded implant

There are two essential fatty acids (EFAs) in human nutrition: alpha-linolenic acid (ALA), an omega-3 fatty acid, and linoleic acid (LA), an omega-6 fatty acid. EFAs are required for the structure of cell membranes and, because they are unsaturated, they help keep membranes flexible. They serve as precursors of eicosanoids, a group of powerful compounds that affect many biological processes, including the aggregation or clumping of blood platelets and the constriction of blood vessels. EFAs also help maintain the barrier of the skin and are involved in cholesterol metabolism [(7)]. Flaxseed (binomial name: *Linumusitatissimum*) contains a mixture of fatty acids. It is rich in polyunsaturated fatty acids, particularly ALA, the essential omega-3 fatty acid (57%), and linoleic acid (16%), the essential omega-6 fatty acid. These two polyunsaturated fatty acids (PUFAs) are essential for humans, that is, they must be obtained from the fats and oils in foods because our bodies cannot make them [(8,9)].

MATERIALS AND METHODS

Commercially pure titanium (CpTi) rods (30 cm in length and 5 mm in diameter) were machined to form 96 screw-shaped implants by using lathe machine. Each screw was 8 mm in length (5 mm threaded and 3 mm smooth) and the diameter was 3.5 mm in the threaded part and 4mm in the smooth part. Implants were divided as follows:

1. Dual-threaded group (32 implants).
2. Single-threaded coated with grinded flaxseed group (32 implants).
3. Single-threaded (control) group (32 implants).

Thirty-two adult male New Zealand white rabbits ranging in weight from 1.75 – 2.75 kg were used in this study. All animals were radiographed prior to surgery to ensure that the bone of the tibia was sufficient to accept the diameter and length of the implants. General anesthesia was induced by intramuscular injection of Ketamine hydrochloride 50 mg/ml (1 ml/kg body weight) and Xylazine 20 mg/ml (1 ml/kg body weight). The skin of both tibiae was shaved and cleaned. All instruments used in surgical procedure were autoclaved and the surgical operation was performed gently under sterile condition. Incision was made on the lateral side of the rabbit's leg; skin, fascia and muscles were reflected to expose the tibia bone.Drilling was performed intermittently by Micro-engine with continuous saline irrigation. Three screws were implanted in each rabbit; the dual-threaded and coated implants were placed in right tibia (Figure 2) while the control one was placed in left tibia (Figure 3). Coating of the implants was done by dipping technique.

Figure 2: The dual-threaded and the coated implants in the right tibia

Figure 3: Control implant in the left tibia

Suturing of the muscles was done with absorbable catgut suture followed by skin suturing with silk suture. An X-ray was taken immediately after the operation to ensure that the implants were properly inserted in their positions (Figure 4).

Figure 4: Immediate radiograph after implantation

The rabbits were divided into four groups according to the healing periods (2, 4, 6 and 8) weeks. At the end of each healing period, eight rabbits were sacrificed; histological, mechanical and radiographical tests were performed for implants.

For histological test, the rabbits were scarified and both tibiae were exposed. The bone was cut about 5 mm away from the head of the implant with normal saline cooling to prepare the bone-implant specimen that was prepared as decalcified section. Histometric quantification was carried out using a light microscope (Olympus, Tokyo, Japan) at a magnification (x2.5) with grid eyepiece with a good located anatomical reference to measure the width of thread and the distance between two opposing threads. Osteoblast, osteocyte and osteoclast counting was performed in five histological sections for each animal and in five microscopic fields at 40x magnification.

Mechanical test was performed by using torque meter with a range of (0-200 N.cm) which was applied into the slits of implant's head and an anti-clock wise movement was applied to measure the torque force required to unscrew the implants.

Radiographic examination was doneafter each healing period to assess bone healing around each implant.

The resulting data were statistically were statistically analyzed by One-way Analysis of Variance (ANVOA), Student t-test and Least Significant Difference (LSD) test.

RESULTS

All animals recovered well after surgery and they moved normally within one week with no weight loss which indicates that they tolerated the implant well. At sacrifice, no signs of gross infection, tissue reaction or any other negative clinical observations were noted around the implant site.

Radiographically, there were no gross changes in the tibial architecture with no areas of radiolucency between implant and adjacent cortical bone.

Histological analysis (Hematoxylin and Eosin stain)

Control group

At 6 weeks duration, new bone surrounds the apex of thread and bone trabeculae coalesce with basal bone (Figure 5).At 8 weeks duration, large marrow space filled most of thread and the immature bone was replaced by new bone (Figure 6).

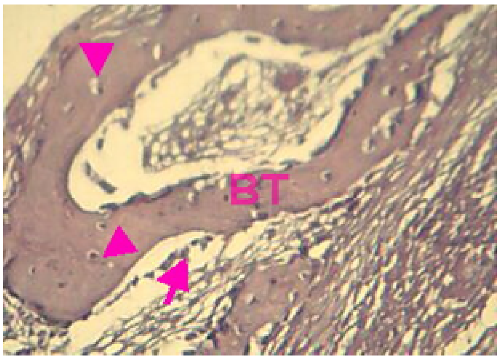

Figure 5: View for thread in control at 6 weeks shows bone trabeculae (BT), osteocytes (arrows heads) and osteoblast (arrow) H&E x20

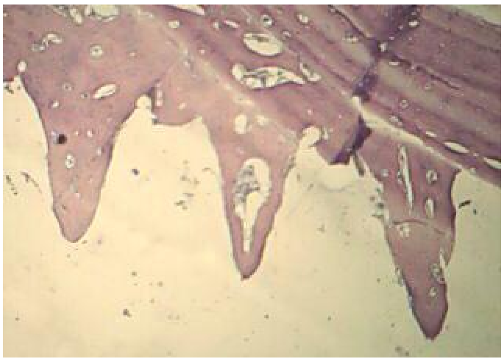

Figure 6: View for threads in control at 8 weeks. H&E x4

Dual-threaded implant group

At 6 weeks duration, new trabeculated bone filled most of thread (Figure 7). At 8 weeks duration, mature bone with osteocytes and Haversian canals were shown (Figure 8).

Figure 7: View for thread of dual-threaded implant at 6 weeks duration H&E x20

Figure 8: View for thread with mature bone fordual-threaded implant at 8 weeks duration H&E x20

Implant with Flaxseed group

At 6 weeks duration, cartilage was surrounded by seed particles and the thread sided by new bone. Then, cartilage was degenerated and replaced by new bone which surrounds the threads (Figures 9, 10and 11).At 8 weeks duration, immature bone filled the thread and occupies the implant bed. The immature bone shows irregularly arranged osteocytes and Haversian canals (Figures 12 and 13).

Figure 9: View shows cartilage (CAL) surrounded by flaxseed particles (arrows) for coated implant at 6 weeks. H&E x40

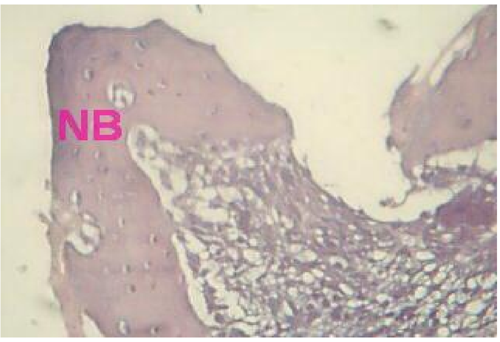

Figure10: View for thread sided by new bone (NB)for coated implant at 6 weeks.H&E x20

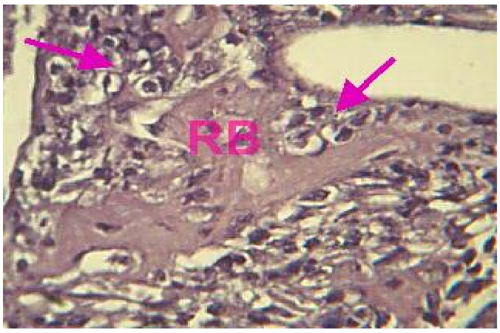

Figure 11: View shows replaced new bone (RB) by degradation of cartilage (arrows)for coated implant at 6 weeks. H&E x40

Figure 12: Immature bone filled the thread for coated implant at 8 weeks.H&E x20

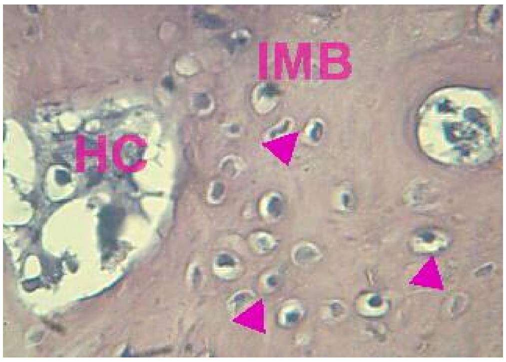

Figure 13: View for immature bone (IMB) shows irregular arranged osteocytes (arrow heads) with Haversian canal (HC)for coated implant at 8 weeks.H&E x40

Radiographical evaluation

The result of radiographic evaluation appeared that there were no gross changes in the tibial architecture with no areas of radiolucency between implant and adjacent cortical bone in all specimens for radiographical examination (Figure 14).

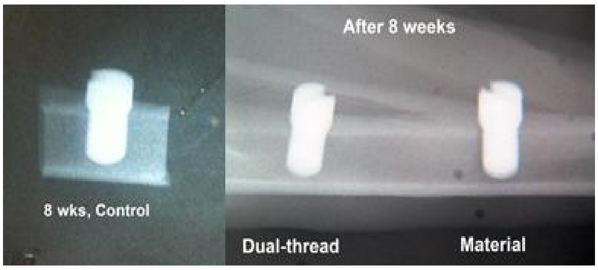

Figure (14): Radiograph of dual-threaded implant, implant with material and control after 8 weeks

Statistical analysis findings

The results illustrate the data of histological measurement for thread separation (µm), thread width (µm), bone cell count (osteoblast, osteocyte, and osteoclast) and the mechanical torque removal values (N.cm^2).

(Table 1) illustrates statistics of Histometric and Mechanical Parameters at different groups distributed in different periods with comparisons significant. The results show the followings:

1. Thread separation decreased with increment of the period of implantation.
2. Thread width increased with increment of the period of implantation.
3. Osteoblast cell count recorded high values at 4 and 6 weeks for all groups.
4. Osteocyte cell count recorded a high value at 6 weeks for all groups.
5. Osteoclast cell count recorded a high value at 4 weeks for control and dual-threaded groups.
6. Torque values increase with increment of the period of implantation and recorded the highest values at 8 weeks for all groups.

Table 1: Descriptive statistics of Histometric and Mechanical Parameters at different groups distributed in different periods with comparisons significant

Parameters	Periods	Group	Control				Dual				Coated			
		No.	Mean	S.D.	F-test	P-value	Mean	S.D.	F-test	P-value	Mean	S.D.	F-test	P-value
Thread Sep.	2nd w	5	14.2	1.64	15	0.000 HS	11	0.71	12.2	0.000 HS	12	0.71	59.1	0.000 HS
	4th w	5	14.4	1.34			11	0.71			9.4	0.89		
	6th w	5	13	0.71			9.8	0.45			7.6	0.55		
	8th w	5	10	0.71			8.4	1.14			6.6	0.55		
Thread width	2nd w	5	13.2	1.3	145.8	0.000 HS	22.4	1.82	70.5	0.000 HS	8.6	1.14	65.05	0.000 HS
	4th w	5	16.8	0.84			27.2	1.92			12.2	1.48		
	6th w	5	20.6	0.89			33.6	2.19			19	1.41		
	8th w	5	26.6	1.14			41.8	2.86			23	2.74		
Osteoblast	2nd w	5	6	2	22.9	0.041 S	8.6	0.89	85.3	0.000 HS	12.2	3.35	18.71	0.000 HS
	4th w	5	11.4	0.89			14.2	0.45			25.8	3.77		
	6th w	5	11.8	1.48			14.2	0.45			20.8	3.03		
	8th w	5	7.4	0.55			10.2	0.84			14.6	2.41		
Osteocyte	2nd w	5	1	0.71	130.1	0.000 HS	3	0.71	132.6	0.000 HS	4.2	0.45	135.4	0.000 HS
	4th w	5	5.4	1.14			8.6	0.55			12.8	1.3		
	6th w	5	11	0.71			10.6	0.89			15	1		
	8th w	5	6.6	0.55			10.4	0.55			15	1		
Osteoclast	2nd w	5	0.8	0.45	13.3	0.000 HS	3	1	10.5	0.000 HS	4	1.22	9.33	0.001 HS
	4th w	5	2.8	0.84			1.6	0.55			2.6	0.55		
	6th w	5	1	0.71			1.4	0.55			2.6	0.55		
	8th w	5	0.4	0.55			0.6	0.55			1.2	0.84		
Removal torque value	2nd w	5	5.4	1.14	122.6	0.000 HS	7.6	1.82	321.9	0.000 HS	6.6	2.07	282.3	0.000 HS
	4th w	5	12	2.12			17	2			8.6	0.89		
	6th w	5	18.8	1.1			29.8	2.05			22.6	2.51		
	8th w	5	34	4.18			48	2.74			42	2.74		

(*) HS: Highly Sig. at P< 0.01; S: Significant at P<0.05

DISCUSSION

All animals tolerated the implantation well; no sign of cross infection, tissue reaction or any other negative clinical indications like mobility of the implants were noted around the implants site.

This study discussed different designs of selected implants, single-threaded implant (control), dual-threaded implant and implant coated with grinded flaxseed.

Mechanical test

All implants were stable during healing periods in the sense that they could not be removed with manual force without the aid of the torque gage instrument. Removal torque values were increasing with advancing time and significant differences between different time periods was present.

A comparison between the different implantation periods shows that the minimum torque value was seen in 2 weeks implantation time while the maximum value was observed in 8 weeks implantation time for the studied groups. These increased values can be attributed to progressive bone formation, maturation and bone remodeling around the implant as time. These findings of increased torque values with time were in accordance with previous studies [10, 11].

Histological findings

Dual threaded implant

Dual-threaded implant records the highest mean value in thread width and the mean values of removal torque in proceeding periods; histologically, it shows mature bone at 8 weeks period. These results can be explained that the Implant design features are one of the most important elements that have an effect on implant primary stability and implant ability to sustain loading during or after osseointegration[4]. In addition, increased surface area of dual-threaded implant led to increased bone to implant contact that improved the interaction between them, increased primary stability and provided a better distribution of forces to the surrounding bone.

Implant with Flaxseed

The present results reported that coated implant with Flaxseed show enhancement of osteoblast and its progenitor cells with highest mean value in bone cell count records and illustrated a proliferating cartilage zone. This result can be attributed to that the Flaxseed powder acts as micro roughed area, which maximizes the interlocking between mineralized bone and implant surface. Cells have mechano-receptor properties that can identify whether or not the surface has features appropriate to begin the process of differentiation [12].

The surface roughness of the implants can significantly alter the process of osseointegration because the cells react differently to smooth and rough surfaces. In addition, enhancement of osteoblast and chondroblast by Flaxseed may related to Flax contains of a mixture of fatty acids. It is rich in polyunsaturated fatty acids, particularly alpha-linolenic acid (ALA), the essential omega-3 fatty acid, and linoleic acid (LA), the essential omega-6 fatty acid. These two polyunsaturated fatty acids (PUFAs) are essential for the structure of cell membranes .They serve as precursors of eicosanoids, a group of powerful compounds that affect many biological processes [13]. Normally, osteoblasts attach on the implant surface from first day of implant insertion, but in our results, chondroblasts seemed to be prominent at first period, forming a cartilage as scaffold, then, replaced by bone with presence of active, and proliferative osteoblast cells.

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الخلاصة

الخلفية:ان تقنيات سطوح الزرعات السنية تتطور بسرعة لتحفيز تكونالعظم بشكل أسرع على تلك الأسطح ولزيادة إمكانية تعجيل علاج الزرع. أما الجزء المسنن من الزرعة فيجب أن يصمم لزيادة مساحة الالتصاق السطحية للحصول على استقرار أفضل للزرعة. اضافة لذلك, تم طلاء الزرعة بمادة بذور الكتان لتَحفيز تكونالعظم.

المواد وطرق العمل:ستة وتسعون زرعة مصنوعة من التيتانيوم النقي التجاريقد زرعت في ساق الأرنب وقسمت الى ثلاث مجموعات وهي زرعات مسننة ثنائيا, زرعات مطلية بمادة بذور الكتان المطحونة و زرعات سيطرة. تم تقييم كل مجموعة ميكانيكيا, نسيجيا وشعاعيا بعد انتهاء كل فترة علاج (6,4,2 و8) أسابيع وحللتالبياناتالناتجةإحصائيابإستخداماختبارتحليلالتباين (ANOVA) و اختبار t عند مستوى معنوية 0.05.

النتائج:سجلت الزرعات المسننة ثنائيا أعلى قيم المتوسط في عزم الدوران وأظهرت تكون عظما ناضجا بعد ثمان أسابيع. أما الزرعات المطلية بمادة بذور الكتان فقد أظهرت تحسين الخلايا المكونة للعظم كما أنها الوحيدة التي بينت تكون منطقة غضروفية والتي ستتحلل لاحقا وتستبدل بالعظم.

الخاتمة:نستنتج من النتائج الحالية بأن كل تحوير للزرعات أظهر منافع مختلفة سواء كان ذلك في تحويرات أسطح الزرعات ميكانيكيا (زرعات مسننةثنائيا) أو طلاء أسطحها بمادة بذور الكتان المطحونة.