Histomorphometric analysis of bone deposition at Ti implant surface dip-coated with hydroxyapatite (In vivo study)

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ABSTRACT

Background: Synthetic hydroxyapatite,($Ca_{10}(PO_4)_6(OH_2)$) can directly bond to bones without infection and fibrous encapsulation, thus is regarded as bioactive and biocompatible. The aim of the study was the estimation of microarchitecture bone parameters include bone mass (gm/cm^2) cortical bone width (mm), thread width (mm), marrow space star volume analysis (V*m) and osteoblast, osteocyte cell number.

Materials and methods: Ninety-six (96) commercially pure titanium CpTi) used in this study, (48) implants were coated with HA by dipping coating and (48) implants were used as control. They were inserted in (32) Newzland white rabbits and followed for 2 & 6 weeks. Mechanical torque removal test and histomorphometric analysis of bone microarchiteture were performed for evaluation of osseointegration.

Results: Results revealed, Torque values were increased with advancing time for coated and uncoated groups. And specifically dip coated implant showed high value in comparison to control. Histomorphometric analysis for bone parameters showed highly significant difference in overall contrasted groups of implant in 2nd and 6th week interval.

Conclusion: Dip coating method is an alternative coating technique for dental implant to enhance better bone implant contact and improve osseointegration.

Key words: Hydroxyapatite, implant surface coating dip-coating histomorohometric analysis. (J Bagh Coll Dentistry 2013; 25(2):70-75).

INTRODUCTION

Titanium has been widely used as implant materials because of their highly biocompatible properties with relatively low modulus, good fatigue strength, and formability, machinability and corrosion resistance. In addition, hydroxyapatite has been added to tatinum surface as a coating material on orthopaedic and dental implants.(1) Adding hydroxyapatite to titanium has allowed combining the strength of the metals with the bioactivity of the ceramics.(2) Hydroxyapatite has helped to promote more rapid osseointgration, this includes direct bony growth and early mineralization at the interface, as well as equivalent or higher bone implant bond strengths and percentage bone contact at the implant interface compared to uncoated titanium implants.(3)

Dip coating: it is an alternative method used in orthopaedic to coat bioactive ceramics such as hydroxyapatite. Dip coating used to modify the surface of the implant material and to creat a new surface with totally different properties with respect to the substrate.(4)

Bioceramic- coated metallic prostheses and implants by dipping method were shown to display increased levels of biocompatibility when placed in the body environment and these thin coatings were also shown to placed in the body environment and these thin coatings were also shown to impede the corrosion and undesired metal ion transfers from the implant itself .(5) Calcium hydroxyapatite biomaterial of choice in coating application. Dip coating techniques can be described as a process where the substrate to be coated is immersed in a liquid and then withdrawn with a well-defined withdrawal speed under controlled temperature and atmospheric condition.(6) The coating thickness is mainly defined by the withdrawal speed, by the solid content and the viscosity of the liquid .(7)

The aim of the study was the estimation of microarchitecture bone parameters include bone mass (gm/cm^2) cortical bone width (mm), thread width (mm), marrow space star volume analysis (V*m) and osteoblast, osteocyte cell number.

MATERIALS AND METHODS

Materials

-Hydroxyapatite powder (Merck, Darmstadt, Germany)

-P_2O_5

-Ethanol Solvent

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-CpTi implants from friatec AG com. (modified and machined to sdiameter of 3.5mm and length 8mm.
-Torquemeter
-Formalin 10% (Tedia, U.S.A).
-Formic acid 10% (Batch No. 28380, England).
-Ethanol alcohol 96%.
-Xylol (Analar, U.K.D).
-Paraffin wax (Analar, U.K.D).
-Haematoxylin and Eosin stain (H&E) (Dako,U.S.A).
-Microscopical glass slides and covers (Sail-China).
-Canada balsam (Batch No.10862501, European Union).
-Optical microscope (Olympus / 542037, Japan).

Methods

Dip coating was applied on (48) implants for each healing intervals. The rest (48) implants used as control. Suspension for HA dip-coating was prepared using 0.2 gm of P_2O_5 as a thickning material, which were dissolved in the ethanol solvent provide the gel.

Surgical procedure was performed to place the implants in suitable size holes in the rabbit tibia, X-ray were taken immediately after surgery to ensure that the implants were properly inserted in their position; sacrification of the animals was done after 2&6 weeks.

RESULTS

All implants at the day of sacrifice were found stable in the bone, they could not be moved with manual force and there were no detectable peri-implant defects at the coronal aspect of any implant screw after 2, and 6 weeks of healing periods. The amount of growing bones around the screw implants head was more for the coated than the uncoated one, and in some cases overhang bone deposition were seen over the head of the screw as clearly seen in Figure 1.

The result of radiographic evaluation appeared that there were no gross changes in the tibial architecture, no areas of radiolucency between implant and adjacent cortical bones in all specimens for radiographical examination as shown in Figure 2.

Mechanical testing

The highest mean torque value was recorded for the CpTi implant dip-coated with HA which was (14.22, 27.73 N.cm) for 2 and 6 weeks respectively of implantation when compared to the uncoated implant. (11.51, 18.44 N.cm) as shown in table 1.

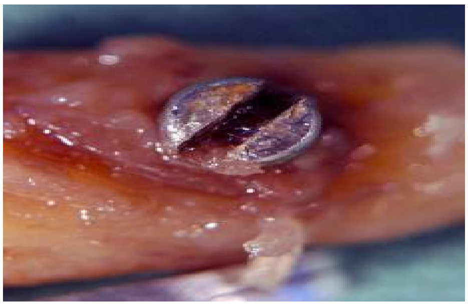

Figure 1: Photograph for implant coated with HA by dip coating method for 6 weeks duration. Notice overhang bone matrix over implant head.

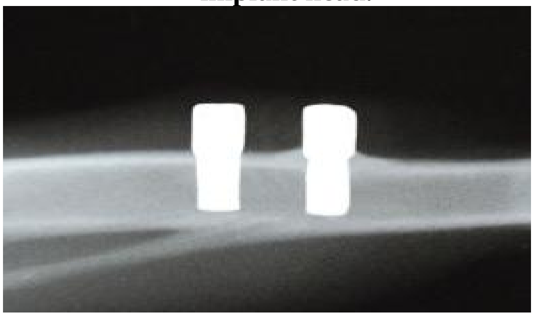

Figure 2: Conventional radiographic view of Ti implant coated with HA by dipping method for 6 weeks duration shows cortical bone bounded the implant coronal protion and radiopaque field around the implant (C) uncoated and (HA) coated with hydroxyapptiate by EPD method.

Histomorphometric analysis

In general the mean value of all histomorphometric parameters were higher at the coated implant than the uncoated ones.

As shown in table (2) the highest mean value of bone mass was obtained at implant dip-coated with HA (0.42gm/cm^2) at 2 weeks duration when compared with the uncoated one (0.37gm/cm^2). After 6 weeks of implantation, there were an obvious increase in the mean value of bone mass (0.54gm/cm^2) the least value was (0.43gm/cm^2) obtained with control group.

Width measurment of bone thread in mm was illustreated in table (3). At 6 weeks duration of time, the highest mean value of bone width was (0.79mm) compared with thread width at uncoated implants (0.24mm) with 95% confidence of intervals for means.

Table (4) shows the mean value of cortical bone width was higher at the coated implant (2.83,3.60mm) at 2 and 6 weeks duration of time respectively, while the mean value of cortical bone width was the least with control group (1.87,2.33mm) respectively.

The mean value of osteoblasts cell number (OST.No,) were obtained at implants dipcoated with HA was (6.25 cells/mm^2) at 2 weeks duration. After 6 weeks of implantation, the mean value was (5.75 cell/ mm^2) as shown in table (5).

Table (6) illustrates the summary statistics for osteocyle cells number (OSC.No.) at implant sites.

The mean value of (OSC.No.) of coated implant was (2.5,6 cells/ mm^2) at 2 and 6 weeks duration of implantation when compared with the uncoated ones (2.25,5.5 cells/ mm^2) respectively.

The mean value of marrow space star volume (V*) at 2 weeks duration was (513.25V*) compared with the uncoated ones (870V*). The highest mean value of V* was noticed at uncoated implant (376.75V*) as shown in table (7).

For exploration the actual significant differences among all probable pair wise of any two group's contrasts, Least significant difference (LSD) method were applied and their outcomes (table8).

DISCUSSION

The interaction between implant materials and the physiological environment play mainly at the interface. Surface topography and chemistry are the most important characteristics that affect cell behavior on artificial materials and that at the end, pilot the entire tissue intergration process.[8] Due to the similarity with the inorganic components of bony structure, synthetic hydroxyapatite. [Ca_{10} (Po_4)6 $(OH)_2$] was one of the first material used to coat metals , so metal implant are coated by bioactive ceramics such as hydroxyapatite increase the growth rate of tissue within the pores and to prevent corrosion.[9]

Histomorphometric measurement is a representative test in study the nature of implant tissue surface and has been used by several authers to evaluate bone- implant-interface. The method supply infomation in trabecular width as well as on its distribution and on organization of trabcule in marrow space .[10] parameter measurement[10] were higher in the coated implant than the uncoated and all the parameter increased with time.

After 6 weeks of implantation there was an obvious increase in the mean value of bone mass, width of bone thread and cortical bone width at implant sites of all coated implants. these findings show maturation in bone – implant interface resuiting in increasing bone function at the implant site and improving osseointegration.[11]

Mean value of marrow space star volum (V*) were measured in 2 and 6 weeks of implantation, the result showed the highest value of (V*) was recorded in the uncoated implant in 2 and 6 weeks duration in comparison with coated with implant, this result related to the more and faster deposition of bone trabeculae more in thickness that decrease V* value in coated implant.[12] For the number of osteoblast and osteocyte cells, Osteoblast cells number was obtained as a highest in all coated group compared to uncoated group which showed lowest number of osteoblasts. Mean value of osteoblast cell number was higher in 2 weeks of all coated implant but in 6 weeks showed a markdly decrease in number.

Cell counting revealed that most of surviving osteoblasts cells are settled on the surface site of coated implants because later in 6 weeks duration, more formation and maturation of woven bone so most osteoblast forming bone become entrapted within the matrix as osteocytes cell .[13] In 6 weeks period, the number of osteocyte slightly decreased which indicate the maturation of bone after replacement of woven bone by mature trabcular bone with less number of osteocyte .[13] Focusing in the result obtained in this study, histomorphometric result support the mechanical result, This finding is in agreement with work of Nkenke et al.[14]

As a conclusion; dip coating method is an alternative coating technique for dental implant. Histomorphometric analysis for bone mass measurement, cortical bone width, thread width and marrow space star volume (V*) showed to be significantly high in overall groups.

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Table 1: Summary statistics for removal torque test in different studied and suggested of coated materials treated along two weeks and six weeks measured continuously

Torque- test										
Period	Material	Groups	N	Mean	S.D.	S.E.	95% Confidence Interval for Mean		Min.	Max.
							Lower Bound	Upper Bound		
After (2) weeks	HD (Dip)	Control	4	11.51	1.07	0.53	9.81	13.2	10	12.5
		Coated	4	14.22	0.94	0.47	12.73	15.71	13.75	15.63
After(6) weeks	HD (Dip)	Control	4	18.44	0.81	0.4	17.15	19.72	17.5	19.38
		Coated	4	27.73	0.46	0.23	27	28.46	27.29	28.13

Table 2: Summary statistics for Bone mass indicator distributed by the different techniques Coated in the 2nd and 6th periods of times (per weeks)

Experiments	N	Mean	S.D.	S.E.	95% Confidence Interval for Mean		Min.	Max.
					Lower Bound	Upper Bound		
HD-2nd w.	4	0.42	0.01	0.01	0.40	0.44	0.41	0.44
Control-2nd w.	4	0.37	0.00	0.00	0.36	0.37	0.37	0.37
HD-6th w.	4	0.54	0.02	0.01	0.52	0.57	0.52	0.56
Control-6th w.	4	0.43	0.03	0.01	0.38	0.48	0.41	0.47

Table 3: Summary statistics indicator for Thread. Wid.mm distributed by the different techniques Coated in 6th weeks periods of times

Experiments	N	Mean	S.D.	S.E.	95% Confidence Interval for Mean		Min.	Max.
					Lower Bound	Upper Bound		
HD-6nd w.	4	0.79	0.01	0.00	0.77	0.80	0.78	0.80
Control-6th w.	4	0.24	0.03	0.02	0.19	0.30	0.20	0.28

Table 4: Summary statistics for Cortical Wid. indicator distributed by the different techniques Coated in the 2nd and 6th periods of times (per weeks)

Experiments	N	Mean	S.D.	S.E.	95% Confidence Interval for Mean		Min.	Max.
					Lower Bound	Upper Bound		
HD-2nd w.	4	2.83	0.05	0.02	2.76	2.91	2.80	2.90
Control-2nd w.	4	1.87	0.25	0.12	1.47	2.26	1.60	2.20
HD-6th w.	4	3.60	0.08	0.04	3.47	3.73	3.50	3.70
Control-6th w.	4	2.33	0.12	0.06	2.13	2.53	2.20	2.50

Table 5: Summary statistics for OST. No. indicator distributed by the different techniques Coated in the 2nd and 6th periods of times (per weeks)

Experiments	N	Mean No./ mm^2	S.D.	S.E.	95% Confidence Interval for Mean		Min.	Max.
					Lower Bound	Upper Bound		
HD-2nd w.	4	6.25	0.96	0.48	4.73	7.77	5	7
Control-2nd w.	4	5.25	0.96	0.48	3.73	6.77	4	6
HD-6th w.	4	5.75	0.96	0.48	4.23	7.27	5	7
Control-6th w.	4	5.25	0.96	0.48	3.73	6.77	4	6

Table 6: Summary statistics for OSC. No. indicator distributed by the different techniques Coated in the 2nd and 6th periods of times per weeks

Experiments	N	Mean No./ mm^2	S.D.	S.E.	95% Confidence Interval for Mean		Min.	Max.
					Lower Bound	Upper Bound		
HD-2nd w.	4	2.5	0.58	0.29	1.58	3.42	2	3
Control-2nd w.	4	2.25	0.5	0.25	1.45	3.05	2	3
HD-6th w.	4	6	0.82	0.41	4.7	7.3	5	7
Control-6th w.	4	5.5	0.58	0.29	4.58	6.42	5	6

Table 7: Summary statistics for V*m indicator distributed by the different techniques Coated in the 2nd and 6th periods of times (per weeks)

Experiments	N	Mean	S.D.	S.E.	95% Confidence Interval for Mean		Min.	Max.
					Lower Bound	Upper Bound		
HD-2nd w.	4	513.25	24.94	12.47	473.56	552.94	480	540
Control-2nd w.	4	870	8.16	4.08	857.01	882.99	860	880
HD-6th w.	4	250	24.49	12.25	211.02	288.98	220	280
Control-6th w.	4	376.75	36.82	18.41	318.16	435.34	330	420

Table 8: Multiple Comparisons for the studied indicators By (LSD) among overall pair wise of the studied parameters of bone microarchitecture at two and six weeks intervals.

(I) groups	(J) groups	Bone mass	Thread. Wid.mm	Cortical Wid.	OB. No	OCS. No	V*m
		Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
HD-2nd w.	FN-2nd w.	0.000	-	0.000	0.000	0.000	0.000
	HEF-2nd w.	0.032	-	0.099	0.000	0.000	0.000
	Control-2nd w.	0.000	-	0.000	0.174	0.660	0.000
	HA-6th w.	0.000	-	0.650	0.730	0.000	0.000
	HAD-6th w.	0.000	-	0.000	0.492	0.000	0.000
	FN-6yh w.	0.001	-	0.402	0.492	0.000	0.000
	HEF-6th w.	0.000	-	0.000	0.009	0.000	0.000
	Control-6th w.	0.605	-	0.000	0.174	0.000	0.000
Control-2nd w.	HA-6th w.	0.000	-	0.000	0.305	0.000	0.000
	HAD-6th w.	0.000	-	0.000	0.492	0.000	0.000
	FN-6yh w.	0.000	-	0.000	0.046	0.000	0.000
	HEF-6th w.	0.000	-	0.000	0.000	0.000	0.000
	Control-6th w.	0.000	-	0.000	1.000	0.000	0.000
HD-6th w.	FN-6yh w.	0.000	0.883	0.000	0.174	0.004	0.433
	HEF-6th w.	0.339	0.001	0.000	0.002	0.012	0.000
	Control-6th w.	0.000	0.000	0.000	0.492	0.381	0.000