Electrophoretic deposition of Hydroxyapatite on tapered Ti-6Al-7Nb dental implants: Biomechanical evaluation in rabbits

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ABSTRACT

Background: Advances in implant design have often focussed on altering the micro-topography and chemistry of the implant surface. This study was done to evaluate the effect of Hydroxyapatite (HA) coating on the bond strength between the bone and tapered screw-shaped Ti-6Al-7Nb dental implants.
Materials and methods: Electrophoretic deposition technique (EPD) was used to obtain a uniform coating of Hydroxyapatite on the tapered Ti-6Al-7Nb screws. Structural investigations were carried out on the HA coating surface of the Ti–6Al–7Nb alloy using X-ray diffraction (XRD). The in vivo studies were performed by the implantation of tapered screw-shaped uncoated and coated implants in the tibia of white New Zealand rabbits. To understand the bone-implant interface, biomechanical test was carried out after 2, 6 and 18 weeks healing periods.
Results: The XRD result shows that the ceramic layer consists of highly crystalline form of hydroxyapatite and the crystalline HA belong to characteristic peaks of (211) at 2Θ 31.7º with the hexagonal symmetry. Biomechanical result shows that there was increased mechanical strength (torque value) of bone-implant interface with time, and the greater increase in the torque value was noticed between 6 and 18 weeks. Also the result shows that the tapered implant coated with HA have higher removal torque values than uncoated one in different time intervals.
Conclusion: The electrophoretic deposition (EPD) is a good alternative coating technique for dental implant. HA coated implants demonstrate high torque value and early healing than uncoated implants, which is very important in early loading of the implant.
Keywords: Electrophoretic Deposition, Hydroxyapatite, Tapered Dental Implants, torque. (J Bagh Coll Dentistry 2011; 23(sp. issue):36-41).

INTRODUCTION

The nature of an implant's surface determines its interaction with the body fluids in particular with proteins which, in turn leads to cascades of reactions comprising the body's response to the implant and determining the implant/tissue interface[1]. A key issue for successful osseointegration is the topographical and chemical design of the implant surface [2].

Primary stability is considered a key factor for the clinical success of dental implants. It is determined by the density of the bone at the site, the surgical technique and the design of the implant [3].Tapered implant designs have been introduced in the market in order to improve primary stability. Experimental and clinical studies with insertion torque measurements and/or resonance frequency analysis have demonstrated higher primary stability for tapered implants compared with parallel-walled implants. Tapered implants demonstrate continuously increasing insertion torque due to lateral compression of the bone from the whole implant length during insertion, then stresses would be distributed along the tapered implant surface and not concentrated to a few spots [4,5].

Bioactive coatings can provide an accelerated healing of bone when compared with an implant surface without bioactive coating also it reduce release of titanium ions to the surrounding tissue thus minimizing the perceived risk of a cytotoxic response [6].

Hydroxyapatite (HA), the most important member of the calcium phosphates family, demonstrates a good biocompatibility behavior and encourages the bone formation. These advantageous characteristics lead to the worldwide use of this type of biomaterials for hard tissue applications[7]. Bioactive HA is deposited on surfaces of metallic implants to promote bone formation and enhance implant-bone adhesion. While bone compatibility is provided by the HA coating, the underlying metal possesses good ductility and strength. Deposition of HA coatings has been achieved by a number of methods, including plasma spraying, ion implantation, sputtering, sol-gel coating,

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biomimetic methods, and electrophoretic deposition (EPD) [8].

Electrophoretic deposition of HA on metal substrates has been studied in an attempt to achieve uniform distribution of fine HA deposits. The advantages of this technique are high purity of layers formed , ease of obtaining the desired thickness , and strong layer adhesion to substrate [9].The suitability for the preparation of the complicated shapes, the ability of the thickness control and the low potentially residual stressing are some of the most significant characteristics of the EPD method [10].

This work is an attempt to study the effect of the electrophoretic deposition of HA on the osseointegration performance of tapered Ti-6Al-7Nb dental implants.

MATERIALS AND METHODS

Implant preparation

Sixty Iraqi new design tapered screw–shaped implants were machined from Ti-6Al-7Nb alloy using Lathe machine. Each screw machined in diameter about 3.0 mm and have a length about 8mm (threaded part is 5mm and smooth part is 3mm) with 6º tapering angle and pitch height was 0.5mm. The screw have vertical lateral groove (Figure 1). The head of the implant have a slit to fit the screwdriver during insertion and to fit the torque meter during mechanical testing. After that the screws were washed in ethanol in an ultrasonic cleaner for 15 minutes and dried at 100°C.

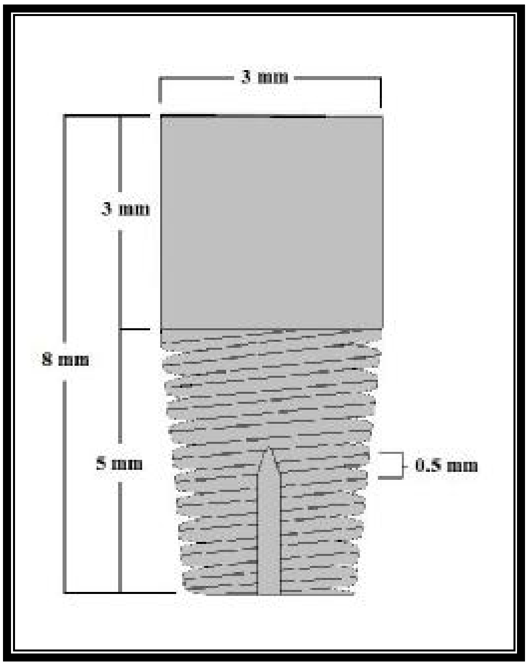

Figure 1: screw implant design.

Electrophoretic deposition

EPD suspension was produced by breaking down agglomerates and uniformly distributing a dispersing agent on the surfaces of the ceramic particles. The particles deagglomeration is carried out by milling and ultrasonic treatment. The HA suspension was prepared by adding HA powder (Particles size 3.8 µm, Merck, Darmstadt, Germany)to the solvent which was the ethanol (l00g/1 liter) in a container over a stirrer without adding any dispersant agent or binder agent. The stirring was continued until a colloidal suspension was obtained. In EPD process application of a DC field causes the charged particles to move forward and deposit on the oppositely charged electrode. The HA powder acquired a positive charge and underwent cataphoresis (deposition on the cathode). Therefore a highly conductive charge surface area anode is used for charging the particles.

Small rectangular pieces of Ti-6Al-7Nb alloy (20 mm x 20mm x 2 mm) and the tapered Ti-6Al-7Nb dental implants were put on the cathode electrode and a piece of the Ti-6Al-7Nb alloy was used as an anode electrode. Both electrodes were connected to the power supply and the meter. The distance between the electrodes was 10 mm. Thirty tapered screws were coated with HA for 5min with 10 V. The coated screws were then sintered at 800 ºC for two hours. Other thirty uncoated tapered screws were passivated for one hour in 28% nitric acid and then rinsed for 5 minutes in each five separated washes of deionized water with ultrasonic cleaner. The screws were then sterilized with gamma irradiation dose of 2.5-3.0 mega rad using gamma cells 220 with Co^{60}source. The irradiation dose is the usual dose recommended for sterilization of medical and surgical materials.

X-ray Diffraction

Phase analysis was employed on Ti-6Al-7Nb alloy specimens before and after coating with HA. Phase analysis was studied using XRD. Shimadzu LabX XRD-6000 powder X-ray Diffractometer using Cu Kα radiation was used for this study. XRD patterns were collected for different 2θ values ranging from 20 to 80°.

In vivo study

Thirty adult New Zealand white rabbits of both sexes weighing 2-3 kg were used. The age of the animals was from 10-12 months. Animals were kept in standard separate cages and had free access to tap water, and were fed with standard pellets. The total animals was divided into 3 groups for each healing interval (2, 6 and 18 weeks) each one consist of 10 animals. For each animal two implants (uncoated and coated with HA) were implanted in the tibia starting from the medial to the distal metaphysic.

Anesthesia was induced by intramuscular injection of ketamine hydrochloride 50 mg (1ml/kg body weight) and xylocaine 10% (1ml/ kg body weight). Surgery was performed under sterile condition and a gentle surgical technique. Incision was made on the lateral side to expose the medial side of the tibia then the skin and fascia flap was reflected .By intermittent drilling, and continuous cooling with irrigated saline, holes (1.8 mm in diameter) were drilled with a 10 mm distance between them, enlargement of the holes were made gradually with drills from 2 to 2.5mm. Bone penetration was performed at a rotary speed of 1500 rpm **.**Finally the operation site was washed with saline to remove debris from the drilling site.The sterilized implants were placed in the bed, using screw driver that fit the screws slit and screwed until the screw thread was completely introduced into the bone tissue and then checked for stability and rinsed with saline. Suturing of muscles was done with absorbable catgut followed by skin suturing with silk suture. The operation site was sprayed with local antibiotic (oxytetracycline spray) .Finally, long acting systemic antibiotic oxytetracycline 20% (0.7 ml/1kg) was given to the animals after the surgery.

Biomechanical test

The animals were anesthetized with the same type and dose that used in the implantation procedure. Incision was made at the lateral side of the tibia then muscles and fascia were reflected to expose the implants. After that, the muscles were removed to expose the entire tibia. The stability of implants was checked by the ends of two hand instruments. Tibia was supported firmly while performing mechanical test to prevent any movement. A torque removal test was done by engaging the screw driver of the torque meter (Sturtevant richmont torque product, model F 80-1-0) into the slit in the head of the implant to determine the peak torque necessary to unscrew the implant from its bed.

RESULTS

X-ray Diffraction analysis

The XRD patterns of Ti-6Al-7Nb specimen coated with HA by electrophoresis deposition method and heat treated at 800^{o}C in comparison with uncoated specimen shows in Figure 2. The pattern of uncoated Ti-6Al-7Nb specimen shows strong line of αTi(002) and (101) at 2θ 38.42 and 40.17 respectively while the βTi mainly(110) at 2θ 38.48 was overlap by prominent peak of αTi(002) at 2θ 38.42.The XRD results of HA coated specimen were fitted to those data reported for the hexagonal Ca5(PO4)3(OH) (JCPDS-ICDD file # 09-0432), Ca10(PO4)6(OH)2 (JCPDS-ICDD file # 00-001-1008) and hexagonal Ca3(PO4)2 (JCPDS-ICDD file # 09-0169).The strongest lines in the XRD pattern are (211), (112), (300), (202) and (312) at 2θ 31.77, 32.19, 32.9, 34.04, and 48.1 respectively, appeared markedly higher than other HA peaks. The strongest (211) peak corresponding to crystalline hydroxyapatite (P63/m) belonging to the hexagonal symmetry. The pattern also showed the diffraction intensity of TCP (315) plane at 2θ 37.85.

Biomechanical test

The descriptive statistics for removal torque values are demonstrated in Table 1. After 2 weeks of implantation the torque value that was needed to remove the coated screws (mean value 12.21 N.cm) was higher than that needed to remove the uncoated Ti-6Al-7Nb alloy screws with mean value 8.63 N.cm. After six weeks of implantation the mean values needed to remove the screws coated with HA was 23.08 N.cm. After 18 weeks of implantation, there was an obvious increase in the means value of the torque force that was needed to remove the implants. The mean torque values for the screws coated with HA coated was 42.29 N.cm. Minimum and maximum values of original data with 95% confidence interval for means were included to represent the real domain of the expected torque.

Finally, Figure 3 illustrates the summery of the torque mean values of uncoated and coated implants. This figure revealed that the torque value varied between different healing period and between coated and uncoated implants in the same healing period.

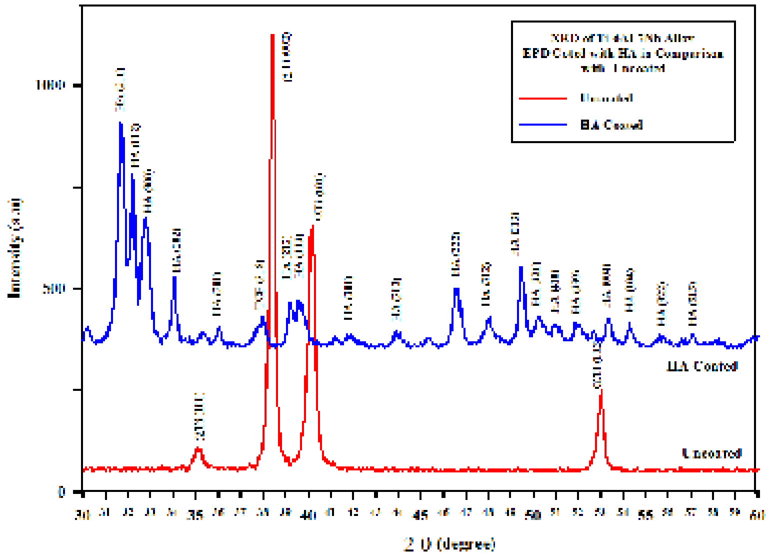

Figure 2: X-ray diffraction patterns of HA coated Ti-6Al-7Nb specimen in comparison with the uncoated specimen.

Table 1: Statistical analysis of removal torque value of coated and uncoated implants at different time intervals.

Time intervals	Groups	N	Mean N/cm	S.D.	S.E.	95% Confidence Interval for Mean		Min.	Max.
						Lower Bound	Upper Bound		
2 weeks	Uncoated tapered screw	10	8.63	0.70	0.22	8.13	9.13	7.50	9.80
	Tapered screw coated with HA	10	12.21	1.22	0.39	11.34	13.08	10.50	13.90
6 weeks	Uncoated tapered screw	10	14.51	0.80	0.25	13.94	15.08	13.20	15.80
	Tapered screw coated with HA	10	23.08	2.27	0.72	21.45	24.71	20.30	26.50
18 weeks	Uncoated tapered screw	10	29.61	1.81	0.57	28.31	30.91	27.00	32.50
	Tapered screw coated with HA	10	42.29	2.13	0.67	40.77	43.81	38.50	45.00

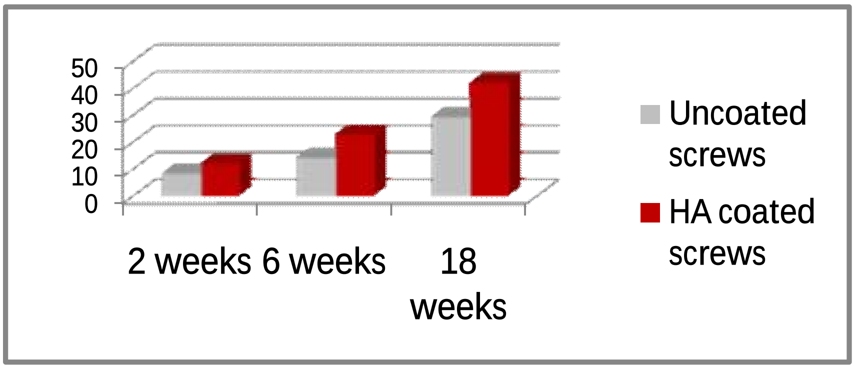

Figure 3: The removal torque values for coated and uncoated implants after different period of implantation.

DISCUSSION

X-ray Diffraction analysis

The result of the present study indicate the crystalline hydroxyapatite belong to characteristic peaks of (211) at 2θ 31.7° with the hexagonal symmetry. The minor trace of the secondary phase (β-TCP) was also observed. The apparition of β-TCP can be attributed to the Ca-deficient nature of the HA resulting in the production of secondary phases post sintering, at temperatures, starting as low as 700°C [(11)]. The XRD result shows that the ceramic layer consists of highly crystalline form of HA, since narrower peaks are indicative of a more crystalline HA (larger crystallite size) whereas broad peaks represent lower levels of crystallinity.

Biomechanical test

Initial stability of implants was achieved in this study because of tapered implant designs and the size of the holes created in the bone smaller than the diameter of the implant which results in a surgical fit. The holes were made 2.5 mm while the screws were 3 mm diameter.

The removal torque was used in this study as an indicator for the presence of osseointegration and as a test for the mechanical property of bone-implant interface, because the torsion appears to be primarily probing the interface mechanics. This technique was used in several clinical and experimental investigations which suggest that removal torque is a useful parameter when studying and comparing screw-shaped implants [12-15].

The mechanical testing results indicated that the surface macrostructure affected the retention of the implants in the bone and the force needed to unscrew the implant from the bone was increased with implantation time. This leads to increased interfacial shear strength with time, which in turn resulted in better stress transfer from the implant to the surrounding bone, a more uniform stress distribution between the implant and bone, and lower stresses in the implant [12,16]. In this study it has been shown that there was increase in removal torque values with time. It has been suggested that this increase depends on an increasing bone-to-metal contact with time as a result of a progressive bone formation and remodeling around the implant during healing, which substantially improved the mechanical capacity[17]. Also Regarding the relation of time to the removal torque Robert et al, 1984 and Branemark et al, 1998, stated that beside an increase in bone to metal contact, also the time dependant increase in removal torque, may reflect change in the structure of bone located close to the implant. As after insertion of an implant, a poorly organized woven bone with low strength is formed at the interface [18,19].

A comparison between the different implantation periods in this study shows that the minimum torque value was seen in 2 weeks implantation time while the maximum value was observed in the 18 weeks implantation periods. The greater increase in the torque value was noticed between 6 and 18 weeks. The torsional strength started to increase after 4 weeks of unloaded healing and there was a significant increase with time during the initial 16 weeks [20]. This large increase in torque values between 6 and 18 weeks may be related to the maturation of woven bone to lamellar bone, which takes 6 weeks in rabbits as revealed by Sennerby et al in 1992[21].

This study showed that the HA coated implants produced a significant increase in the torque value when compared to the uncoated ones within different time periods. The implantation of a CaP coated implant in porous bone will lead to a higher success rate than using an uncoated implant because of earlier and possibly a better fixation with bone, a gap between the coating and the bone can be bridged [22,23].Al-Mudarris, in 2006 showed a significant difference between biomimetically HA coated and uncoated Ti-6Al-7Nb implants even after 18 weeks[14]. The application of CaP coating on metal implants that combines the mechanical strength of the metal with the ceramics favorable biological properties. In addition to the interfacial bonding to bone introduced by CaP coatings on dense metal implants, applying a porous structure can even further advance bony integration, whereby mechanical interlocking may also enhance the integration process [24].

The partial dissolution of hydroxyapatite makes the surrounding fluids rich in calcium and phosphate ions, which seems to induce the precipitation of "bone-like" apatite on implant surface. The release of calcium and phosphate ions followed by precipitation of "bone-like" apatite can trigger cellular differentiation and consequent bone formation (apatite plus osteoblast cells)[25], and would perhaps explain why HA-coated implants tend to exhibit faster osseointegration than uncoated implants.

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