

Evaluation of nano surface modification on CPTi dental implant using chemical method: mechanical and histological evaluation

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ABSTRACT

Background The application of nanotechnology to biomedical surfaces is explained by the ability of cells to interact with nanometric features. The aim of this study was to consider the role of nanoscale topographic modification of CPTi dental implant using chemical etching method for the purpose of improving osseointegration.

Materials and methods: Commercial pure titanium rod was machined into 20 dental implants. Each implant was machined in diameter about 3mm, length of 8mm (5mm was threaded part and 3mm was flat part). Implants were prepared and divided into 2 groups according to the types of surface modification method used: 1st group (10 implant) remained without nano surface modification (control), 2nd group include (10 implant) etched with 15N H₂SO₄ and 30% H₂O₂. Surfaces were characterized by scanning electron microscope (SEM), Xray diffraction (XRD), atomic force microscope (AFM), thickness measurement for the invitro experiments. While for invivo part tibia of 5 white new Zealand rabbits were chosen as implantation sites. The tibia of each rabbit received two screws. Biomechanical test was performed to understand the bone-implant interface, after two weeks healing periods. Implants from 4animals were tested for the torque required to remove the implant from the bone and the other one animal was prepared for histological examination.

Results and Conclusion: For in vitro results, scanning electron microscope showed that the chemical etching of Ti substrate becomes highly porous and has surface consisting of nanosized pits. Removal torque means value after 2 weeks of implantation mentioned that, there was a gradual increase in the removal torque mean values as a follow (M±SD): 12.625(N.cm) ± 0.517, 30.500(N.cm) ± 4.071for machined surface(X), nano chemically etched (X1) respectively. In addition, the histological analysis showed improved quality of bone in response to the nano modified screws, that the chemically treated implants shows trabeculated thread.

Keywords: Removal torque; Titanium screw; Rabbit tibia; acid etching. (J Bagh Coll Dentistry 2015; 27(3):8-14).

INTRODUCTION

Interaction between the biomaterials surface and osteoblasts is strongly associated with the biocompatibility of dental implants ⁽¹⁾. The connection of dental implants and bone is of great interest to research on biomaterials and a significant number of studies have been carried out to investigate improvements of the bone/biomaterials interface ⁽²⁾.

Titanium (Ti) is largely used as an implant biomaterial due to its mechanical properties and high in vitro and in vivo cytocompatibility, allowing direct bone-to-implant contact ⁽³⁾. In an attempt to increase the amount and quality of the bone-implant interface, surface treatments such as surface machining, acid etching, electro polishing, and anodic oxidation, sandblasting or plasma-spraying. These methods induce chemical modifications associated with alterations in surface topography ⁽⁴⁾.

It has been shown that methods of implant preparation can significantly affect the properties of the surface and subsequently the biologic response that occur at the surface ⁽⁵⁾. Chemical treatments, such as acid etching of the Ti implant

surfaces, are of particular interest because they accelerate the healing process around implants. Treatment of Ti with a mixture of H₂SO₄/H₂O₂ produces a surface that affects events of in vitro osteogenesis, resulting in an increase in bone-like nodule formation, as well as more bone-to-implant contact in vivo ⁽⁶⁾.

Further, recent studies have demonstrated the use of chemical oxidation to create reproducible nanopatterns on the commonly used biocompatible metals such as Ti and Ti alloys. By simply immersing the Ti-based material in an etching solution made by mixing concentrated sulfuric acid (H₂SO₄, a strong acid) and aqueous hydrogen peroxide (H₂O₂, an oxidant), it is possible to create a reproducible sponge-like network of nanopits on the surface. These surfaces have been demonstrated to have beneficial effects on both initial and subsequent osteogenic (bone-forming) events in vitro ⁽⁶⁾.

Nanoporous surfaces topography tend to favor the proliferation and differentiation processes, acting directly on the selective adhesion of osteoblastic cells on the surface, which can accelerate the healing process around implants ⁽⁷⁾.

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MATERIALS AND METHODS

1. Sample preparation

Commercial pure Titanium grade 2 plates were used as the substrate for surface modification, these plates were cut from sheet by a seissor in to small square pieces specimen of (16×17×0.25mm) length, width and thickness respectively. These plates had a mirror polished surface from the source. Debris and contamination were removed by ultrasonic cleaning in ethanol bath for 15 minutes, followed by distilled water for 10 minutes, then the plates dried at room temperature. These plate samples were divided into 2 groups as follows: no surface modification (mirror polish from the source) (X), nanosurface modification using chemical etching method (X1). The plates treated with a mixture consisting of equal volumes of 15N H₂SO₄ and 30% aqueous H₂O₂ for 4 hours under continuous agitation according to the results obtained from pilot study.

2. Pilot study

In pilot study CPTi plates treated with a mixture of equal volumes of 15N H₂SO₄ and 30% aqueous H₂O₂^(8,9) for 2 times (2hr. and 4hr.). The plates placed in a beaker containing the oxidative mixture for either 2h or 4 h at room temperature under continuous agitation using magnetic stirrer (2MLH, India). Then the plates washed with distilled water under continuous agitation for 15 minutes to eliminate acid residues and then dried at room temperature to be ready for testing.

3. Examination of surfaces:

a. Examination of nanomodified surfaces

X-Ray phase analysis: The structure of control (X) and chemically etched (X1) were examined by X-ray diffractions using Cu K α target radiation. The 2θ angles were swept from 20- 80° in step of one degree.

b. Surface roughness measurements

The surface roughness of control and chemically etched plates were examined using scanning probe microscope (AA3000 Angstrom Advanced Inc., USA).

c. SEM analysis

The morphology of control and chemically etched plates were imaged using scanning electron microscope (SEM Tescan vega 111, Czech). This device was operated at 30.0kV to determine the size and shape of pits in the micrographs for chemically etched sample.

4. Implant preparation

CPTi rod (USA) was used for preparation of implants. Twenty screws were machined from

CPTi rod; using Lathe machine with cutting head coated with titanium carbide, the length of the screw was 8mm (5mm was threaded and 3mm was flat) pitch height was 1 mm, and 3mm in diameter. They have a slit in the head of 1.5mm depth and 1mm width to fit the screwdriver and torque meter during insertion and removal and slit in the center of smooth part. These screws were thoroughly ultrasonicated in ethanol bath for 20 min to remove the debris and contamination and then dried at room temperature.

Out of the 20 screws, ten screws were nanosurface modified using chemical etching method according to the result obtained from pilot study the implants treated with mixture consisting of equal volumes of 15 NH₂SO₄ and 30% aqueous H₂O₂ for four hours under continuous agitation. Then implants washed with dH₂O under continuous agitation for 15 minutes to eliminate acid residues and dried at room temperature. The remaining 10 implants were left as machined surfaces.

The screws were sterilized with gamma irradiation dose of 1000rad using gamma cell 220 with a CO60 source. The energy of the used radiation was 1.25 MeV (Million Electron Volts) with a dose rate of 90.4 rad/min and 80 cm distance between sources of radiation and dental implant, the total time is 23.49 minute. All implants were kept in their airtight plastic sheets till the operation day.

5. Surgery

Five adult male Newzealand white rabbits weighing 1.75-2Kg were used. Animals had free access to tap water and were fed with standard pellets and carrot. They were left for 10 days in the same environment before surgical operation. Subcutaneous one dose of 10 mg Ivermectin injection was given to ensure parasite free animals. The total animals No. 5 were divided into 2 groups, First group include 4 animals for mechanical test (torque removal test). While 2nd group include 1 animals was sacrificed for histological study. Four implants were implanted in the tibia, two implants (one control and one chemical treated) were implanted in the right tibia and 2 implants (one control and one chemical treated) were implanted in the left tibia consequently starting from the medial to distal metaphysis for each animal.

All instruments were autoclaved at 121 C° and 20 bars for 30 minutes before operation. The required dose of anesthesia and antibiotic was calculated by weighting each rabbit in a special balance for the animals. The animals were anaesthetized with a combination of Ketamine

(25mg/kg) and Xylazine (17.5mg/kg) intramuscularly. Prior to surgery 20% of Lidocaine was injected locally (1cc/1cm) into the tibia metaphase. Surgical operation was performed under sterile condition and gentle surgical technique. Prior to surgery, the legs were shaved, washed and decontamination with a mixture of iodine and 70% ethanol.

The tibia metaphysics was exposed by incision through skin, fascia and periosteum. The flat surface on the anteriomedial aspects of the tibia was selected for implant placement. By intermittent drilling, and continuous cooling with irrigated saline holes (1.8 mm in diameter were drilled with 10 mm distance between them, enlargement of the holes were made gradually with drills from 2.2 to 2.6mm. The operation site was washed with saline to remove debris from drilling site.

The sterilized implants were placed in the bed, using screw driver that fit the screw slit was completely introduced into the bone tissue and final screwing was done with torque meter (approximately 10N.Cm). Then the implant checked for stability. Suturing of muscles was done with absorbable catgut, Followed suturing of skin with skin suture. The operation site was sprayed with local antibiotic (oxytetracyclin spray). Postoperatively care was performed by giving local and systemic antibiotic (20 mg/Kg oxytetracycline) for 3 days after surgery; the animals were followed daily for 2weeks.

6. Mechanical testing (Torque test)

The same surgical instruments and anesthetic solution used in the implantation phase. For healing interval after 2weeks four animals were used for mechanical testing using removal torque meter .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. A torque removal test was done by engaging the screw driver of the torque meter into the slit in the head of the implant to determine the peak torque necessary to unscrew the implant from its bed.

One animal was used for histological test with optical microscope. It was injected with an overdose of anesthetic solution. Cutting of the bone around the implant was performed using a disk in low rotating speed hand piece with normal saline cooling. Cutting was made about 5 mm away from the head of the implant to prepare a bone- implant block for histological study. Bone-implant blocks were immediately stored in 10% freshly prepared buffered formalin.

RESULTS AND DISCUSSION

1. X-ray Diffraction of Samples

X-ray diffraction patterns of untreated, nanosurface modification using chemical etching method shown in **figure 1**. In chemically etched plate, it appeared the same pattern of control but with decreased intensity, and did not show any anatase or rutile peaks and this agrees with **Ji - Hyun Yi et al.**⁽¹⁰⁾

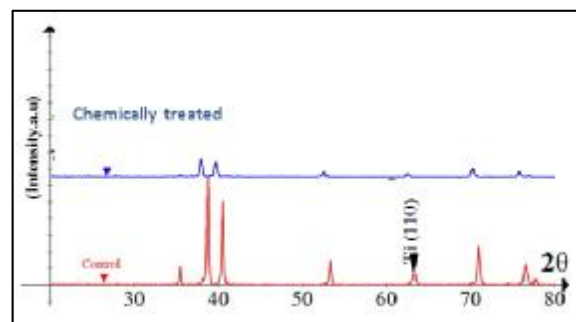


Fig. 1: X-ray diffraction patterns of control and chemically treated plates

2. Nanosurface feature.

Morphological analysis (SEM)

In **Fig. 2 A** the untreated plate showed parallel grooves resulted from machining and polishing with no topographical features, whereas a distinctive texture characterized by network of nano-sized pits appearance was clearly seen on the chemically treated CPTi surface for 4 hour. The average diameter of nanopits was calculated

by take more than one pit and it was approximately 38.5nm **Fig. 2B** which indicated that the use of H₂O₂ with acid etching has the ability to create novel nanostructures of amorphous titanium oxide on the implant surface **Wang et al.**⁽¹¹⁾. The micrograph of the sample that chemically etched for 2 hour as shown in **Fig. 2 C**, it showed that there were changes at the surface at low and high magnification but it

doesn't have the distinctive texture nanopits as the sample that treated for 4 hour.

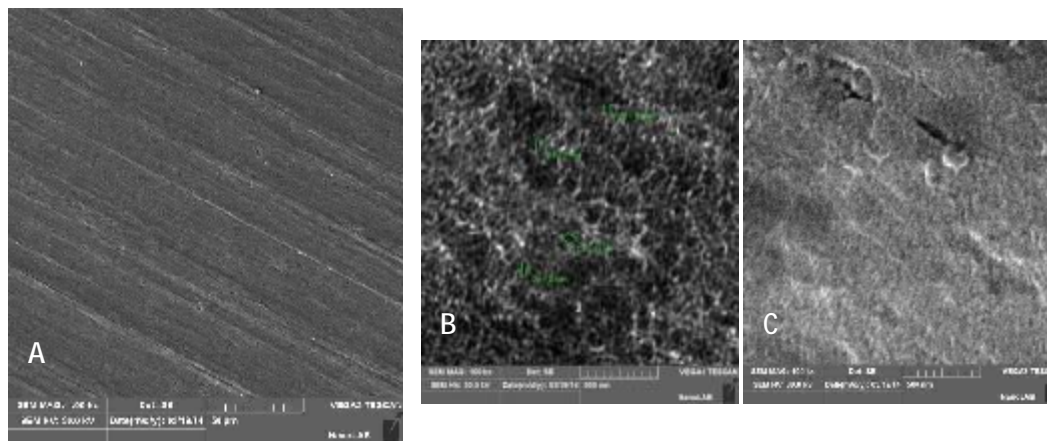


Fig. 2: SEM (A) control (B) chemically etched for 4 hour (C) chemically etched for 2 hour

Nanoroughness surface analysis

AFM topographies of Ti surfaces before and after chemical etching (2 and 4 hour) as shown in **fig (3A, B, C)**, grooves of mechanical polishing were observed on the untreated surface with average roughness 7.37nm as shown in **fig (3 A)**. In contrast, **Fig (3B, C)** establishes that chemical treatment changes the surface topography completely, and the etched Ti surface consists of nano-sized peaks and pits with increased roughness 15.1nm. In chemically treated plate at 4

hours the roughness increase from 13.8 to 15.1nm. It can be observed that the grain size was nanometric in chemically treated Ti plate (**49.24 nm in 2 hour and 61.46 nm in 4 hour**). The chemical etching increases the roughness of the surface lead to increased surface area on the implant and promotes the opposition of bone integration of implants in bone⁽¹²⁾. The summary of surface roughness and average diameter of grain is shown in **table (1)**.

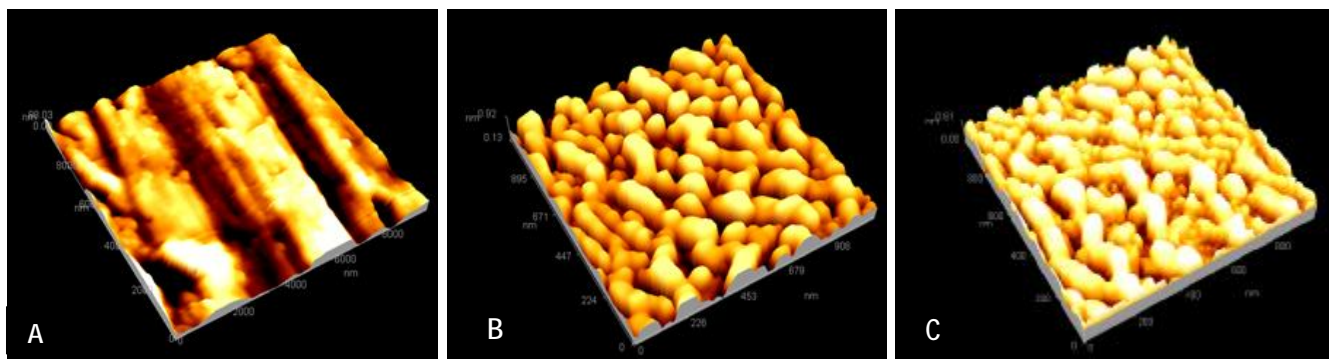


Fig. 3: AFM topographies of cpTi surfaces: (A) control, (B) chemically treated for 2 hour (C) chemically treated for 4 hour

Table 1: Roughness values (nm.) obtained from AFM images for all tested group

Group	Avg. Diameter of grain	Roughness average
Control (untreated)	222.15 nm	7.37 nm
Chemically etched (2 hour)	49.24 nm	13.8 nm
Chemically etched (4 hour)	61.46 nm	15.1 nm

3. In vivo experiments

Clinical observations

All animals tolerated the implantation well after surgery and moved normally within one week. One animal suffered from tibia fracture and was replaced with another rabbit. At sacrifice, no sign of gross infection, tissue reaction, or any other negative clinical observations were noted around the implant sites in any of the animals. 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 weeks of healing periods. In vivo experiments included rabbit as an animal model. This is because of ease of manipulation and rapid bone healing response compared to other models⁽¹³⁾.

The tibial sites in the rabbit were chosen to mimic the clinical situation, and since the dimensions of this bone correspond well with human alveolar space. The morphologic characteristics of the rabbit tibia allow for implant fixture to engage cortical bone at its coronal

aspect and marrow in the apical area⁽¹⁴⁾. 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⁽¹⁵⁾. Gradual size of drill until reaches the final diameter of 2.6 mm in an attempt to increase compression and thereby the stability of the implant during insertion⁽¹⁶⁾.

4. Mechanical testing

Descriptive statistics of removal torque values of machined surface dental implant, nanosurface modified dental implant by chemical etching after 2 weeks of implantation are shown in **Table (2)**. In this interval, a high torque value was needed to remove the chemically treated screw (mean value of **30.500 N.cm**) and the lowest torque value was needed to remove the control (machined screw) with mean value of **12.625 N.cm**.

T-test was used to significant compare between means in this study. (**Table 3**) showed a highly significant difference among two groups.

Table 2: Removal torque mean values of all tested groups after 2 weeks of implantation

Group (Treatment)	N	Mean ± S.D.	Range
Control	8	12.625 ± 0.517	12-13
Chemical	8	30.500 ± 4.071	25-35

Table 3: T-test for the comparison between two tested group

Groups	P-value	Sig.
Control & chemical etched	0.0001	HS
HS highly significance		

This study indicates that rough acid etched implants achieve greater resistance to reverse torque removal than machined surface implants increased surface roughness may enhance the mechanical interlocking between the macromolecules of the implant surface and the bone, resulting in a greater resistance to compression, tension and shear stress demonstrated which agree with **Richert et al.**⁽¹⁷⁾.

In addition chemical etching with H₂SO₄ and H₂O₂ produced nanofeature which indicated a clear relationship between cell behavior and the morphological properties of the nanotextured Ti-based biomaterials⁽¹⁸⁾. The significance in the difference of removal torque values between machined dental implants and nanomodified chemical etched implants may be due to that nanopits might provide encouraging environment for the progenitor cells to proliferate and differentiate and large surface area.

5. Histological features of implant after two weeks of implantation

Deposition of osteoid tissue at the apex of and the base of thread was shown in machined dental implant as shown in **Fig. (5 A)**. The tabeculated thread was seen in the Ch as showed in **Fig. (5B)**. Osteoblast and osteocyte can be detected as showed in **Fig. (5C)**. This is due to roughness which is of great importance in the bone stimulating.

Histological analysis was used in this study since it is the method of highest reliability to evaluate implant stability that can be performed at any time of the implantation, as stated by **Atsumi et al.**⁽¹⁹⁾. It is clear from the obtained results that no inflammatory reaction was observed during the experimental periods regardless of the type of implant and the duration of the implantation. This is agreed with the results of **Giampietro et al.**⁽²⁰⁾.



Fig. 5-A: Osteoid tissue (OST) deposits at the base of thread H &E ×20.

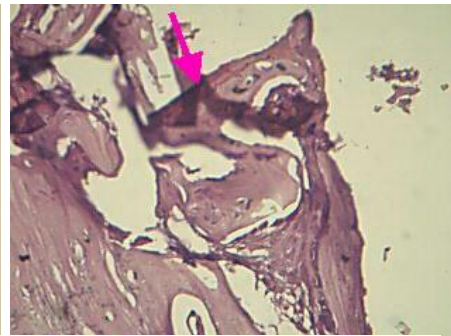


Fig. 5-B: Histological view for hemically etched dental implant shows thread (arrow) H&E×10.



Fig. 5-C: Magnifying view for bone trabeculae (BT) shows osteocytes (arrow head) H&E×20.

As conclusion; chemical method can be considered as an a suitable method for obtaining nanofeature and characterization with high removal torque mean value which was statically significance difference as compared with micron scale morphology. One might suggested that rapid bone formation response to using chemical etching is dependent on better biocompatibility of the material and on the surface topography which greatly affects the histological and biomechanical properties of the interface.

ACKNOWLEDGEMENT

This study was supported by Al-Mustansyria University/ Department of Physics and Veterinary Medicine College.

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الخلاصة

المقدمة: تتوفر زراعة الاسنان طريقة العلاج الفريدة لاستبدال الاسنان المفقودة. وقد تم تحقيق النجاح السريري ليس فقط بسبب المتانة الميكانيكية او الانسجام الحيوي لمادة الزرعة ولكن ايضا بسبب الخصائص الاخرى مثل خصائص السطح.

الهدف: النظر في دور التعديل النانوي لسطح زرعات الاسنان من التيتانيوم النقي التجاري باستخدام الطريقة الكيميائية في تحسين الاندماج العظمي.

المواد و الادوات : ان قضيب التيتانيوم النقي تم تحويله ومكثته الى (٢٠ غرسه). كل غرسه صنعت بقطر ٣ ملم وطول ٨ ملم (خمسة مليمات منها ملولب وثلاث مليمات لمساء).

تم تحضير وتقسيم الغرسات الى مجموعتين وفقا لطرق تعديل السطح المستخدمة: تركت المجموعة الاولى (١٠ زرعات) دون تعديل السطح (نانو السيطرة). المجموعة الثانية (١٠ زرعات) حفرت باستخدام ١٥ ان من حامض الكبريتيك مع ٣٠% اوكسيد الهيدروجين.

ولمعرفة التغيرات التي تحدث على السطح ولمعرفة التحليل الهيكلي تم استعمال فحص انحراف الاشعة السينية, المجهر القوة الذرية, قياس السمك, مجهر المسح الإلكتروني, المجهر الضوئي. أما بالنسبة للدراسة التجريبية على الحيوانات فقد تمت عن طريق زراعة ٢٠ غرسا. اختير عظم الساق ل (٥) من الارانب النيوزلندية البيضاء مكانا للزرعة. وضعت في الساق اليمنى و اليسرى من كل ارنب ملولبين. ولفهم السطح البيئي للعظم و الغرسا اجري الفحص الاحيائي ميكانيكي بعد اسبوعين من مدة الشفاء. اربعة منها لإجراء الفحص الميكانيكي بواسطة فحص عزم التدوير بينما استخدم واحد لأغراض إختبار الأنسجة بالمجهر الضوئي.

النتائج : نتائج الدراسة الخارجية, أظهر مجهر المسح الإلكتروني ان الحفر الكيميائي للقاعدة التيتانيوم اصبحت ذات مسامات عالية ولها سطح يتكون من حفر نانوية الحجم. نتائج عزم التدوير بعد اسبوعين من الزرع, كان هناك زيادة تدريجية في قيم متوسط عزم التدوير كما يلي: 0.157 ± 12.265 , 0.500 ± 30.071 للمجاميع السيطرة والمحفورة كيميائيا على التوالي. وبالإضافة الى ذلك أظهر التحليل النسيجي تحسين نوعية العظم بشكل ايجابي للغرسات المعدلة نانويا. وان الزرعات المعالجة بالطريقة الكيميائية اظهرت تكونا عظميا.