

# Physical and Histological Evaluation of Coated Implant with Nano ZrO<sub>2</sub> after Creation Titania Nanotubes

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## ABSTRACT

**Background:** Contact between implant material and bones must be strong and fast creation, to fulfill these properties appropriate surface modifications must apply on used implants. In this contribution; double surface modifications are applied on Ti-6Al-4V alloy to accelerate osseointegration.

**Materials and methods:** Anodic process is utilized to create titania nanotubes (TNTs) on the screws made from Ti-6Al-4V alloy. These implants were coated with nano ZrO<sub>2</sub> particles. Second modification was annealing anodized screws at 800°C, and implanted in tibiae of nine adult New Zealand white rabbits.

**Results:** Physical and histological consequences of two surface modifications on Ti-6Al-4V alloy screws were studied. Scanning electron microscope (SEM) images shows inhomogeneous distribution of TNTs on screws surfaces. X-ray diffraction (XRD) patterns illustrate the covering of first group samples with ZrO<sub>2</sub> and transformation of Ti to its oxide (Rutile phase) for second group. These pattern shows that TiO<sub>2</sub> had higher crystallinity and larger grain size than ZrO<sub>2</sub>. Atomic Force Microscopy images (AFM) shows the increasing of roughness, grain size and internal diameter of TNTs after annealing process. Coated implant with ZrO<sub>2</sub> at 4 month duration shows threads with newly Haversian canal feature. Annealed implant at same duration shows well developed threads, base of implant illustrates bone trabeculae filled the base of implant bed with active osteoblast cells.

**Conclusion:** Modification of implant's surface produced an improvement of osseointegration in comparison to untreated one.

**Keywords:** ZrO<sub>2</sub>, titanium nanotubes, annealing, osseointegration and electrophoretic deposition. (J Bagh Coll Dentistry 2016; 28(4):89-95)

## INTRODUCTION

Surface modification (within the field of biomaterials) is of special interest since the interaction between the living tissues (including blood and arteries) and implanted devices are mediated by reactions at the surface of the implant; biocompatibility is widely be considered as a surface property <sup>(1)</sup>.

Difference surface modifications have been experienced to Ti and its alloys to enhance bone differentiation and consolidate direct contact between implant material and bones. However for long periods, none of these modifications have produced a durable interface strong enough to support functional loading. Hence, there is specific necessity to develop implants with surface coatings designed to improve bone anchorage through enhanced osseointegration <sup>(2)</sup>.

Attempts to improve the osteoconductivity of medical alloys can be divided into two techniques: coating metallic implants with bioactive materials to accelerate bone formation, and forming rough surface at the macro-level on these implants and the ingrowth of bone results in anchorage of the implants <sup>(3)</sup>.

The integration of the implant into bone takes place largely at the tissue-implant interface. Development of this interface is complex and involves numerous factors. These include not only implant related factors, such as material, shape, topography and surface chemistry, but mechanical loading, surgical technique and patient variables, such as bone quantity and quality, as well <sup>(4)</sup>.

Because of their good combination of mechanical properties and excellent biocompatibility, zircona ceramics are recognized as one of the best biomaterials for dental prostheses <sup>(5)</sup>. This material is chosen in form of nanoparticles to coat Ti-6Al-4V alloy after creation TNTs on its surface.

Annealing process (the second surface modification) has many merits in improving the performance of TNTs as active biomaterial. This process can reduce contamination of species from the electrolyte or organic solvents <sup>(6)</sup>, improved the stability of TNTs samples <sup>(7)</sup>, activated the anodic oxidation layer and the surface becomes bioactive <sup>(8)</sup>.

The aim of this work is investigation the effect of covering TNTs with nano ZrO<sub>2</sub> and annealing these tubes for getting best osseointegration.

## MATERIALS AND METHODS

### 1- Samples preparation

Ti-6Al-4V alloy discs shapes were prepared by using wire cut machine. These samples were

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utilized for characterization purposes. The same material was used to make medical screws with following dimensions: the head diameter was 3.5mm while body was 3mm in diameter. They have a slide in the head of 1.5mm depth and 1mm width. Ultrasonic cleaning bath (Sonomatic/170-2-T80, Germany) with ethanol and acetone of 75:25 wt% ratio was carried out to removing debris and contamination from the fabricated samples.

## 2- Samples characterization

The crystalline nature of the materials was tested by X-ray Diffractometer (XRD) using Cu  $K\alpha$  radiation. Surface morphology of the modified surfaces was examined using Scanning Electron Microscopy (SEM), type (JEOL-JSM-5600).

## 3- Anodic and annealing processes

To create TNTs on screws anodic process was used, the details are mentioned in reference <sup>(9)</sup>. After anodic process; the thermal treatments on samples were performed in a tubular furnace for two hours at 800°C.

## 4- Coating with ZrO<sub>2</sub>

EFD method was used to deposit nano-ZrO<sub>2</sub> material on screws after creation TNTs on their surfaces. Amer et al. <sup>(10)</sup> mentioned deposition details of this material on Ti-6Al-7Nb alloy. The deposition conditions were: Deposition time 3 min, applied voltage 50 volt, collide temperature 25 °C and the distance between electrodes was 1 cm. Sintering of the coated screws was carried out using Carbolite furnace type MTF, England. The treatment was done at 800 °C under inert gas (argon), to prevent oxidation of the specimen. Figure 1 shows untreated, annealed and coated samples.

## 5- Surgical implantations and specimens collection

Nine adult New Zealand white rabbits weighing 1.5-2 kg were used. For each rabbit, two screws were implanted in the right tibia (untreated and annealed), coated screw with ZrO<sub>2</sub> was implanted in left tibia. The details of surgical implantation were mentioned in reference <sup>(11)</sup>. Specimens of bone with different implant samples were subjected to decalcification process and the slides were stained by H&E stain and examine under light microscope.



Figure 1: From left: Untreated, Coated with ZrO<sub>2</sub> and Annealed sample.

## RESULTS AND DISCUSSION

Figure 2 shows XRD pattern of untreated, annealed and coated Ti-6Al-4V alloy. Annealed sample has mixed peaks, first type belongs to titanium and the second one belongs to titanium oxide. Strong lines of XRD profile of annealed alloy with the following Miller indices (hkl): (110), (101), (111), and (211) belong to (Rutile phase) (JCPDS file no. 211276). This reflections indicating that the as-annealed nanotubes layers are crystallized. Inside this pattern there is strong lines with high intensity can be assigned to Alpha phase titanium with Miller indices (101) and (100)

(JCPDS file no.441294). This refers to incomplete transformation to TiO<sub>2</sub> by annealing process.

Strongest reflections peaks for coated sample are (-111) and (111) which could be indexed to ZrO<sub>2</sub> monoclinic phase corresponding to JCPDS file 37-1484. Comparing the values of Full Width at Half Maximum (FWHM) between the dominant peak of annealed and coated samples shows that the grain sizes of the former larger than that of latter. Also, TiO<sub>2</sub> consists of highly crystalline form, since narrower peaks represent higher levels of crystallinity.

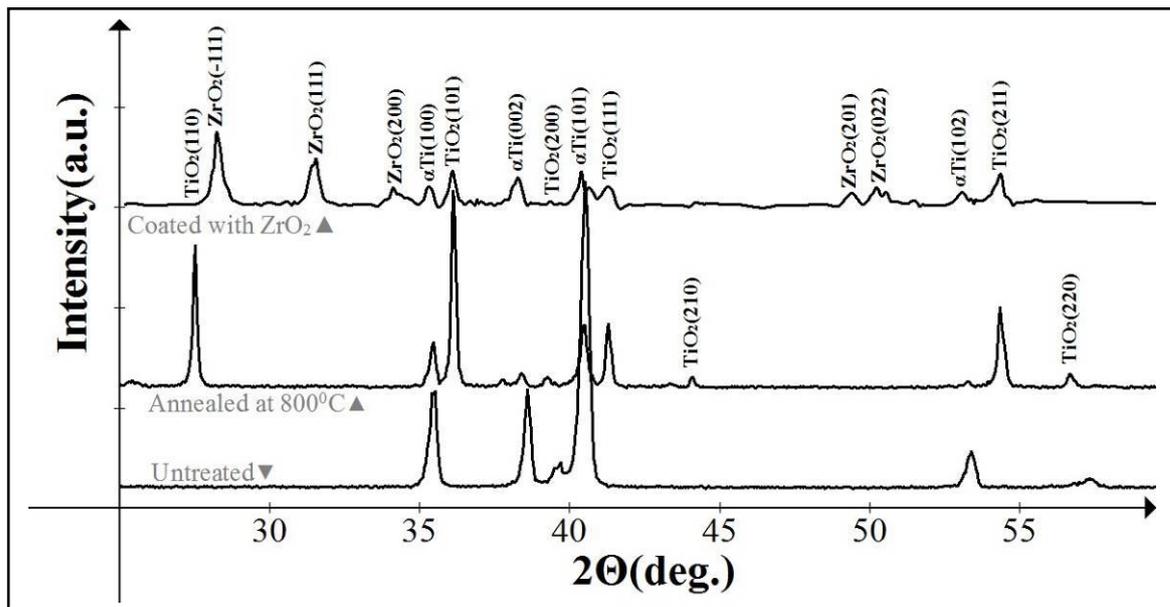


Figure 2: XRD patterns of untreated, annealed and coated samples.

Figure 3(A) shows the SEM image of TNTs which were created on medical screws fabricated from Ti-6Al-4V alloy. These nanotubes are not uniform or have the same length over entire surface; this is might due to the effect of the geometric design of machined screw. It's well known that <sup>(12)</sup>: on the binary  $\alpha + \beta$  type Ti alloys, inhomogeneous nanotube formation takes place. On the surface of this body the arrays of nanotubes formed on  $\alpha$  phase region while the selective dissolution of  $\beta$  phase occurs, leading an inhomogeneous oxide nanotube layer.

EDS spectra of anodized screw are illustrated in figure 3 (B). The concentrations of the elements are listed in table (1); these concentrations did not seem to be directly related to the overall composition of Ti-6Al-4V alloy. Anodized alloy contains 69.3% Ti and 19.077% O which is not close to the stoichiometric composition of TiO<sub>2</sub>. So Ti does not transforms completely to titania phase after anodization. Wt% of vanadium element in table has zero value because of the overlapping between Ti-K $\beta$  line and V-K $\alpha$  line.

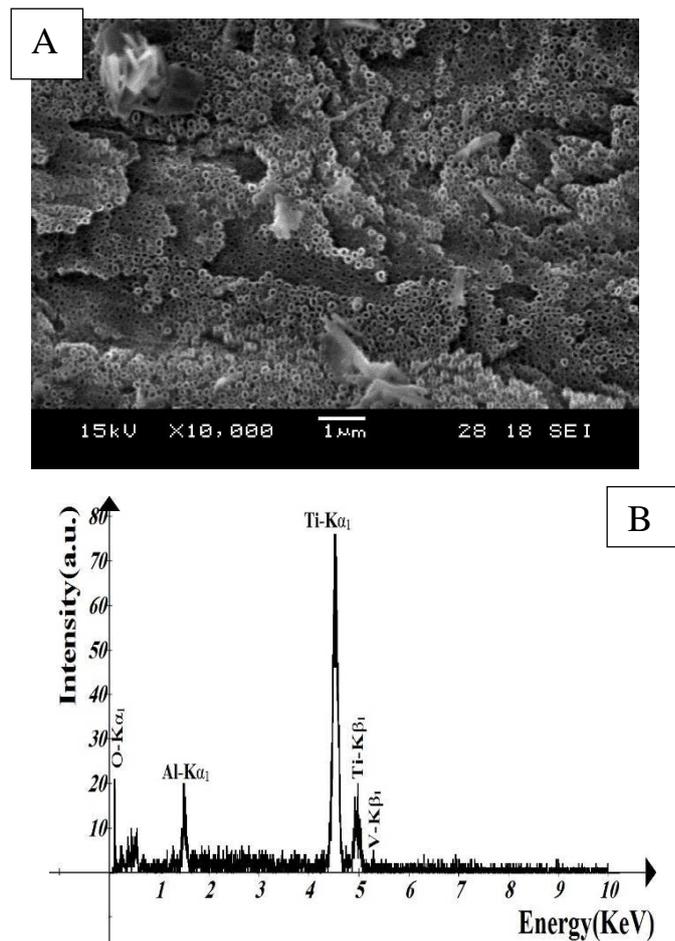


Figure 3. For anodized Ti-6Al-4V. A-SEM image. B- EDS spectra.

**Table 1: Concentration of anodized Ti-6Al-4V alloy elements determined by EDS**

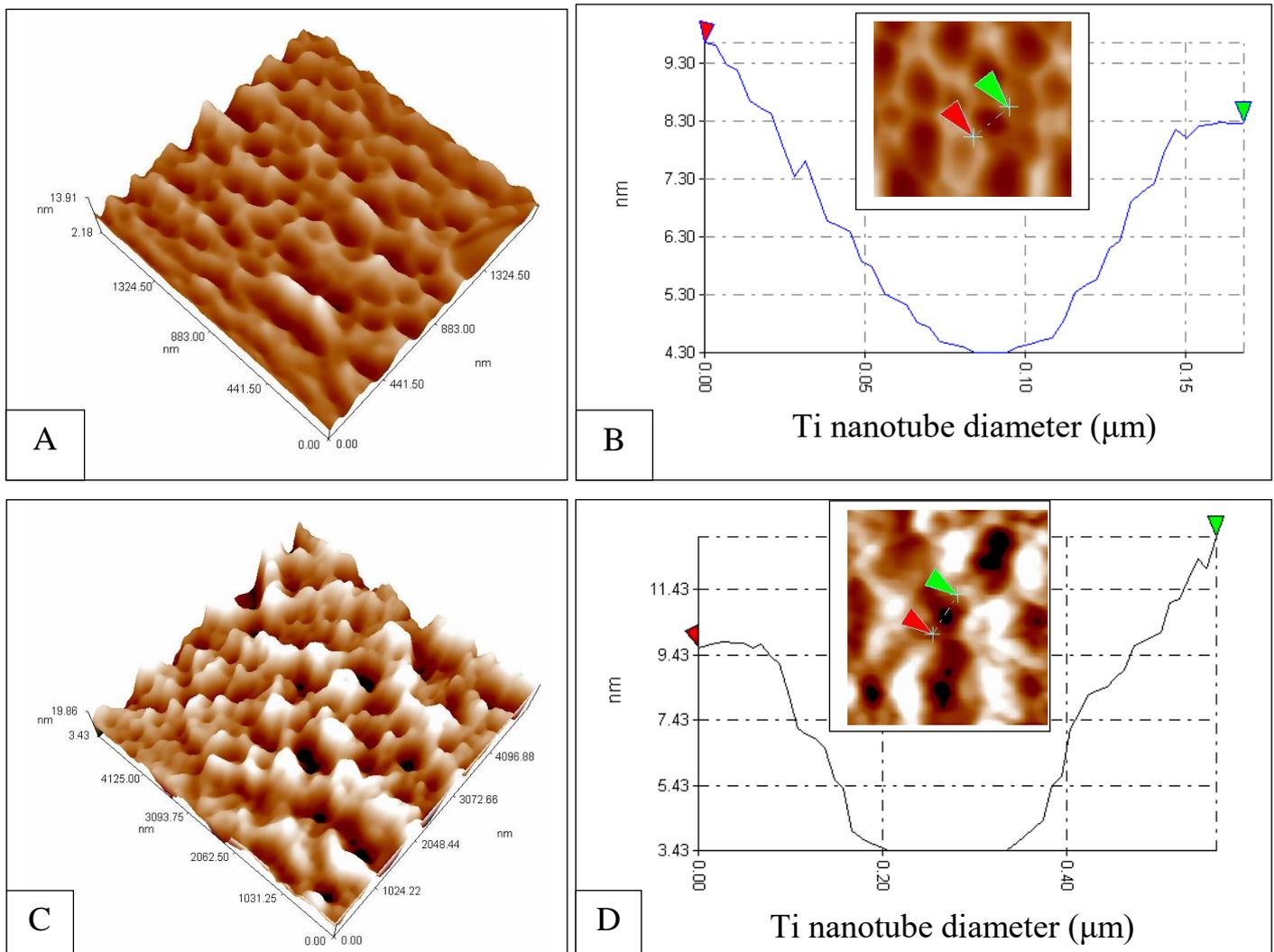
The Element	Atomic number	Energy (KeV) of $K_{\alpha 1}$ lines	wt%	at%
O	8	0.523	7.822	19.077
Ti	22	4.51	85.07	69.297
Al	13	1.487	6.185	8.948
V	23	4.951	0	0

Figure (4-A and C) shows Atomic Force Microscopy (AFM) images for anodized and annealed alloy. This image illustrates the effect of annealing on TNTs. Figure (4-B and C) illustrates upper views of single Ti nanotube on anodize and annealed samples respectively.

Table 2 shows the data of AFM images of anodized and annealed Ti-6Al-4V alloy. These data confirm the increasing of roughness, grain size and internal diameter of TNTs after annealing process. The increasing of roughness has good effect on accelerate osseointegration.

**Table 2: AFM images parameters of anodized and annealed samples**

Sample's type	Roughness Average (nm)	Grain Size (nm)	Internal diameter of TNTs (nm)
Anodized alloy	1.2	96.40	171
Annealed alloy	2.7	171.57	572



**Figure 4: (A),(C): AFM images of anodized and annealed Ti-6Al-4V alloy respectively. (B),(D): Measurement of Ti-tube after anodic and annealing processes respectively.**

Removal torque forces have been used as a biomechanical measure of anchorage or osseointegration in which the greater forces required to remove implants may be interpreted as an increase in the strength of osseointegration <sup>(13)</sup>. Table 3 illustrates the effect of current surface modification to build strength bonding between implants and bone tissue.

**Table 3: Average values of removal torque values of untreated and treated samples after implantation for four months**

Treatment type	Average values of removal torque values (N.cm)
Untreated	40
Anodized and coated with ZrO <sub>2</sub>	55
Anodized and annealed at 800°C	65

**Histological findings**

Untreated implant at 4 month duration shows threads with immature bone feature, base of implant illustrates fine and few trabeculae with active osteoblast cell rimming the bone, figure 5(A,B,C).

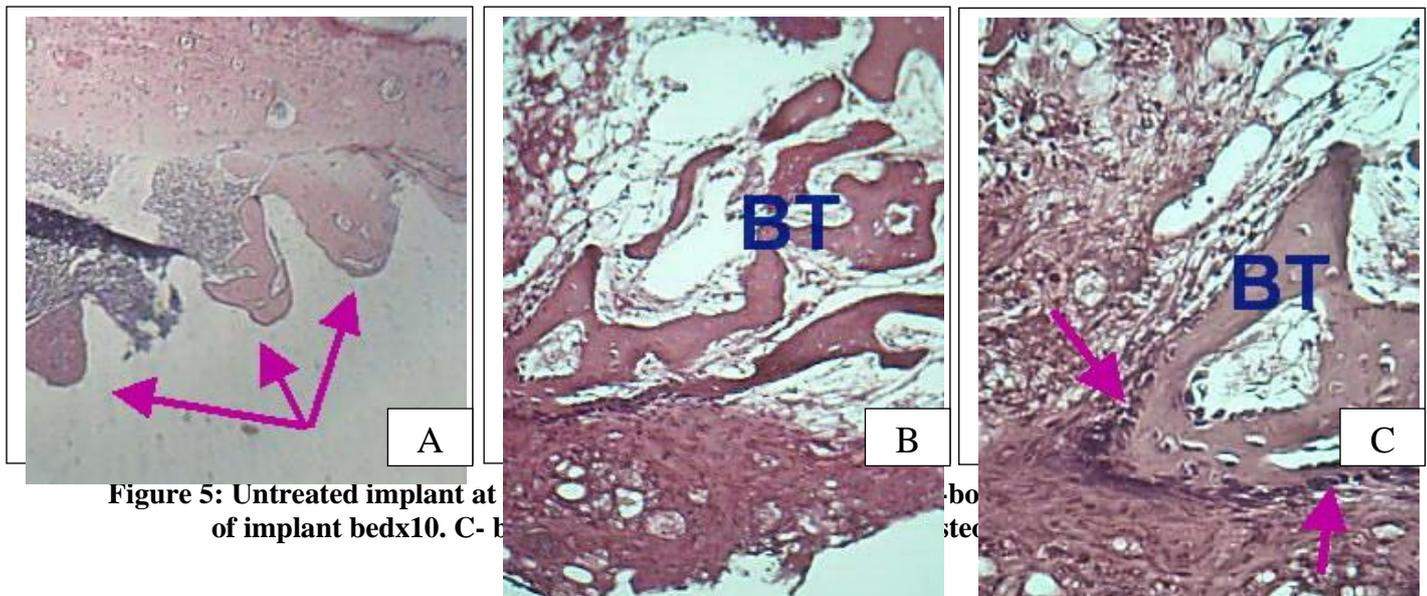
Coated implant with ZrO<sub>2</sub> at 4 month duration shows threads with newly bone formation, base of implant illustrates fine, numerous trabeculae with active osteoblast and osteocyte cells, figure 6(A,B,C).

The enhancement of bone formation at the bone-implant interface has been achieved through the modulation of osteoblasts adhesion and spreading, induced by structural modifications of the implant surface, particularly at the nanoscale level <sup>(14)</sup>.

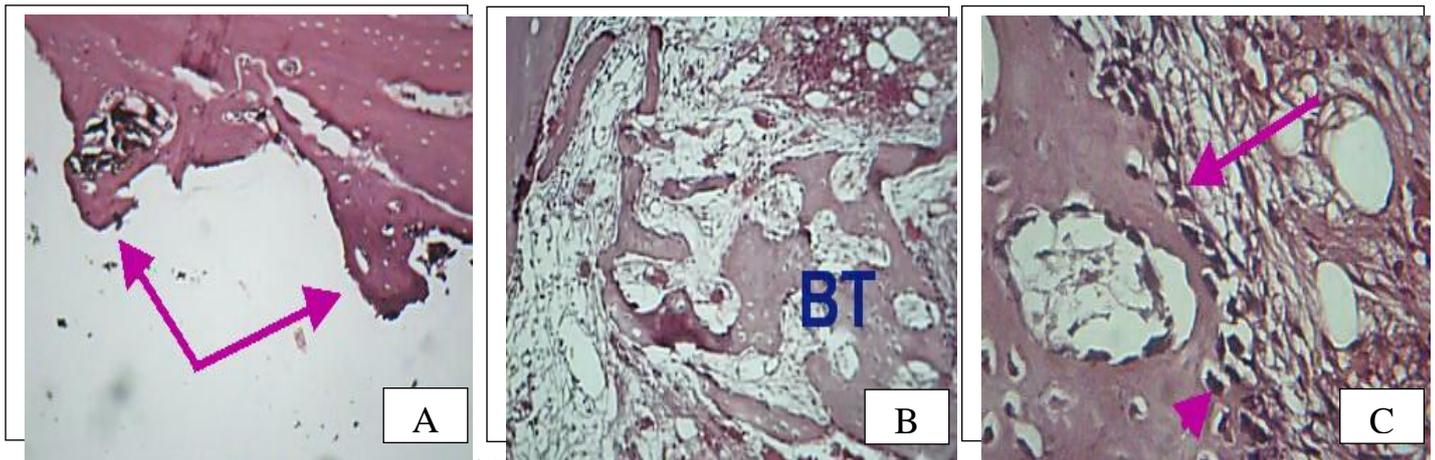
In present results the improvement of the bone-forming activity at the bone-implant interface for coated implant with ZrO<sub>2</sub> is committed to nanoscale features that have the ability to induce the differentiation of stem cells along the osteogenic pathway and because of their ability to differentiate into different types of functional cells, as they possess great potential to restore and regenerate native tissues <sup>(15)</sup>.

For annealed implant at 4 month duration, result shows well developed threads, and the base of implant illustrates bone trabeculae that filled the base of implant bed with active osteoblast cells, figure 7(A,B,C). The present result shows the great potential for the conversion of natural by-product into highly valuable compounds for bio-applications, using a simple and effective valorization process <sup>(16)</sup>. In addition, the improvement in bone-implant interface shows to be more by use of natural biomaterial rather than the synthetic.

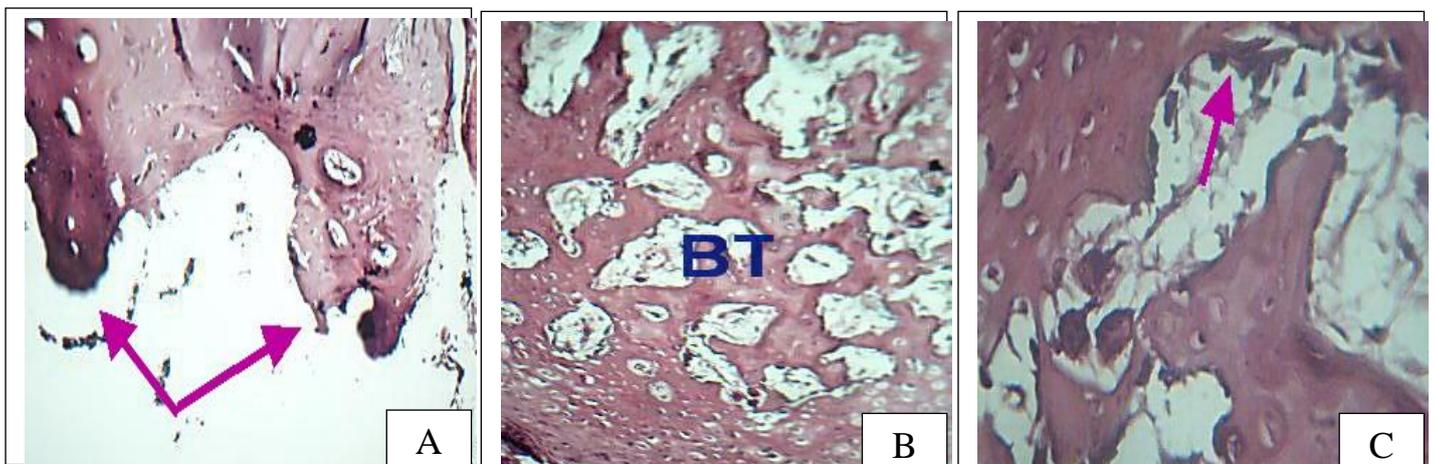
As a conclusion; due to its high roughness and histologic findings that revealed for annealed process, it had superiority in production of active osseointegration, in comparison to others.



**Figure 5: Untreated implant at 4 month duration shows threads with immature bone feature, base of implant illustrates fine and few trabeculae with active osteoblast cell rimming the bone, figure 5(A,B,C).**



**Figure 6: Coated implant with  $ZrO_2$  at 4 month. A- Threads (arrows)x10. B-bone trabeculae (BT)at base of implant bedx10. C- Active osteoblast (arrow); osteocyte (arrow head)x20.**



**Figure 7: Annealed implant at 4 month. A- Well developed threads (arrows) x20. B-bone trabeculae (BT) filled the base of implant bedx10. C- Bone trabeculae rimming by osteoblast (arrow) x40.**

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