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# Histological Evaluation of Pure Titanium Dental Implants Coated with a Mixture of Nano Titanium Oxide and Nano Hydroxyapatite

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## Abstract

**Background:** Dental implant surface characteristics affect the success of implant treatment. Many studies have focused on coating titanium (Ti) implant surfaces with bioactive materials. Bio-active surfaces play a crucial role in enhancing the healing process of the human bone tissue surrounding dental implants.

**Aims of the research**: This study aims to evaluate the histological response and bonding strength of titanium screws coated with a combination of nano-sized titanium oxide ($TiO_2$) and nano-hydroxyapatite (HA) after healing intervals of two and six weeks.

**Materials and methods**: A suspension was prepared using 5.27 g of nano-hydroxyapatite and 2.68 g of nano-titanium oxide. Commercially pure Grade II titanium screws, measuring 3.0 mm in diameter and 8 mm in length, were coated using the dip-coating technique. The presence of the nano-coating on the titanium surfaces was confirmed through X-ray diffraction (XRD) and microscopic analysis. For histological evaluation, some screws were coated with the nano $TiO_2$–HA mixture, while others were coated solely with nano-HA, and both types were implanted into the tibiae of New Zealand rabbits. Peri-implant bone formation was analyzed at two and six weeks post-implantation using a light microscope equipped with a GT-N7100 camera.

**Results:** Optical microscopy revealed a uniform, crack-free coating layer on the titanium screw surfaces. XRD patterns confirmed the presence of both hydroxyapatite and titanium dioxide phases. Histological analysis showed enhanced new bone formation in the group with the $TiO_2$–HA mixture coating, evidenced by early osteoblast proliferation and more advanced bone maturation at both two and six weeks post-surgery, when compared to implants coated only with nano-hydroxyapatite.

**Conclusion:** The implant coating mixture of nano $TiO_2$ & nano HA promotes new bone formation more rapidly than a coating of nano hydroxyapatite alone.

**Key words:** Nano $TiO_2$, nano HA, dental implant coating, histological assessment .

## Introduction

Dental implant is a prosthetic device made from alloplastic material (s) that is surgically inserted into the oral tissues beneath the mucosa and/or periosteum, or directly into the bone, to support and retain fixed or removable dental prostheses. [1].

The fundamental process behind the effectiveness of dental implants is osseointegration, a biological process in which materials such as titanium establish a direct and stable interface with the surrounding bone. After the implant fixture is inserted into the bone, osseointegration begins. Once sufficient integration has occurred, a dental prosthesis such as a crown, bridge, or denture is affixed to the implant. It is essential to allow adequate healing time to ensure proper osseointegration before attaching the final restoration[2,3,4].

Titanium (Ti) is widely used for implants because of its excellent mechanical and chemical properties. Its high biocompatibility is largely due to its resistance to corrosion, which arises from the formation of a stable oxide layer on its surface. This oxide layer notably changes the surface topography of titanium, particularly regarding pore orientation. [5,6]

A widely studied biological apatite is hydroxyapatite (HA), also referred to as calcium hydroxyapatite or simply apatite. HA is an inorganic bioactive ceramic that closely resembles the chemical composition of natural bone. It is considered a bioactive material because it can form strong chemical bonds with adjacent bone tissue. [9] Various techniques have been explored to apply HA and other calcium phosphate-based coatings to metal implants. These include plasma spraying, sol-gel processing, electron beam deposition, and ion beam sputtering. While these methods show promise, they can present difficulties when used on implants with intricate geometries. [10,11,17,18]

The dip coating technique is another method used for applying coatings. It involves immersing the object in a solution and then withdrawing it under carefully controlled temperature and atmospheric conditions. This technique is favored for its simplicity and adaptability to various implant shapes. [12,13]

This study was performed to evaluate the bone-implant interface of pure titanium oxide implants after dipping in a mixture of nano $TiO_2$ and nano HA .

**Materials and methods:**

This study was approved by the Research Ethics Committee of the college of dentistry, Uruk University (Approval No. 232004).

**Sample preparation for an in vitro experiments:** Commercially pure Titanium (grade 2) (OrotigSrl Eu , Italy) was used as the substrate for coating and characterization test. The titanium rod was cut into small circular discs (29mm diameter and 2 mm thickness) by lathe machine (Zaozhuang, Shandong, China) .

**Implant preparation:** Eight screw-type implants were manufactured from commercially pure titanium (Cp Ti) rods (Orotig Srl Eu, Italy) using a lathe machine. Each implant measured 3.0 mm in diameter and 8 mm in length, consisting of a 5 mm threaded portion and a 3 mm smooth section. The implants, designed with a thread pitch of 1 mm, were intended for histological evaluation.

**Preparation of coating solution**: To create the nano-hydroxyapatite (nHA) coating solution, 0.01 g of phosphorus pentoxide ($P_2O_5$, 99.9% purity) was dissolved in 50 ml of absolute ethanol (99.8%). This mixture was stirred continuously and heated at 45°C on a hot plate for 30 minutes. After that, 7 g of nano-hydroxyapatite powder (SkySpring Nanomaterials, USA), was gradually added, maintaining the same temperature, and stirred for an additional 30 minutes. For the combined nano-hydroxyapatite and nano-titanium oxide coating solution, 5.27 g of nHA and 2.68 g of nano-$TiO_2$ powders were blended using the same protocol. All powders were measured precisely using a digital analytical balance with 0.001 g sensitivity (Scaltec, Germany).

**Discs and screws coating**: Titanium discs and screw implants were coated by immersing them in the nanoparticle suspension for 5 seconds, followed by a one-minute withdrawal phase. This dipping cycle was repeated three times to achieve consistent and even coating layers.

**Heat treatment**: To enhance the coating's density and adhesion, the coated specimens were sintered in a tube furnace (Carbolated furnace, BAMFORD, England; Serial No. 3/88/432). Sintering was conducted in an argon atmosphere to prevent oxidation, with optimal results achieved at a processing temperature of 400°C (see Figures 1 and 2).


**Figure (1): CP Ti discs (a) Uncoated, (b) nano HA coating, (c) mixture of nano HA &$TiO_2$nano HA coatng**

**Figure (2): “CP Ti screws coated with mixture of nano HA &$TiO_2$**

**Sterilization of screws:** The screws were sterilized with gamma irradiation at 2.5-3.0 mega rad using gamma cells 220 with CO 60 source (Vishal India)**.**

**<u>Tests performed</u>**

**A. Microscopical examination**: The appearance of each sample for each type of coating (two samples) was examined by using optical microscope at different magnification powers (50 x , 100 x), (Nikon Eclipse ME 600L/441002, Japan)”. Supplied with digital camera type DXM 1200 F. Nikon ACT version 2.62 , 2000 soft ware.

**B. “X-Ray phase analysis**: Phase analysis was applied on CP Ti discs before and after coating with selected materials using (Shimadzu LabX- XRD- 6000) (SHIMADZU, XRD-6000, Japan). The peak indexing was done depending on the JCPDS (joint committee on powder diffraction standards).

**<u>Sample grouping</u>:**

**A.** Control group (4 screws): This group consists of 2 screws for each healing interval (2 weeks and 6 weeks), coated with Nano HA.

**B.** Experimental group (4screws): This group includes 2 screws for each healing interval (2 weeks and 6 weeks), coated with mixture of nano HA and nano titanium oxide .

**Surgical procedure**: Four implants (2 nano HA coated and 2 mixed nano HA and nano $TiO_2$ coated) were implanted in the tibia of New Zeeland rabbits. Each tibia received one implant coated with nano HA and the other one coated with mix. of nano HA and nano $TiO_2$) consequently starting from the proximal to the distal side for each experimental tibia (see Figures 3 and 4).

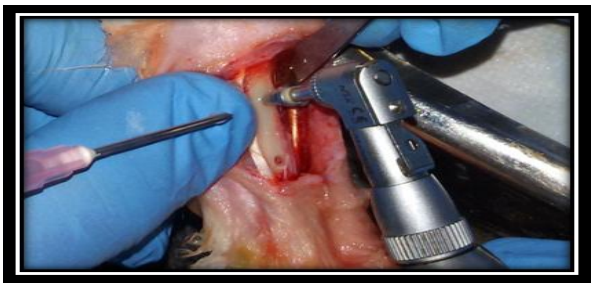

**Figure (3): Hole preparation within tibia of New Zeeland rabbits for screw implantation**

**Figure (4): Screw insertion (distance 1cm between 2 screws)**

**Histological testing**

**Methods.** Bone sectioning was done while the animal was anesthetized with (Ketamine 50 mg: HOLLAND, DIAZEPAM 10 mg Iraq, Xylazine 20, Holland). A disc cutter with low rotating speed and vigorous cooling was utilized for sectioning the bone around the implant. Cutting was applied 5mm away from the head of the implant to make bone-implant block for histological assessment. Bone-implant blocks were immediately placed in 10% freshly prepared formalin (Achema, Urope) and allowed to stand overnight for fixation (Figure 5).

**Figure (5): Bone-implant block stored in 10% formalin for fixation**

Following fixation, bone decalcification was performed by dipping the specimens in 10% solution nitric acid (Achema, Urope) (2 to 3 days), the implant was gently eliminated from its bone bed.[14]. the bone- implant block was sectioned horizontally into two parts using a sharp scalpel,[15]. Dehydration of the specimen was applied by dipping it in alcohol with serial concentration (70%, 80%, 90% and absolute alcohol remaining in each dish for one hour). Specimens were passed through two changes of xylene for 15-20 min. Each specimen was placed in a dish of melted paraffin and the dish was kept into a constant-temperature oven regulated to 60°C for 30 minutes. The specimens were molded in the center of paraffin block, and adjusted to microtome where serial sectioning with (4-5) µm of thickness for each section was performed and placed on a slide. Total of 30 sections were made for each coating material in each period of time, the slide was placed in a container having haematoxylin and eosin stain for 10 minutes to stain the tissue". Photomicrographs were captured at 40x magnification using a light microscope equipped with a SAMSUNG GT-N7100 camera.

**Results**

**Optical microscopical findings.** The images revealed a uniform and continuous coating layer of nano-hydroxyapatite (HA) and a composite of nano-HA with nano-$TiO_2$ on the surface of the titanium discs. Notably, the coating appeared intact and free of cracks (Figures 6 and 7).


**Figure (6):** Optical micrographic Nano HA coated disc surface with no cracks in the coating layer

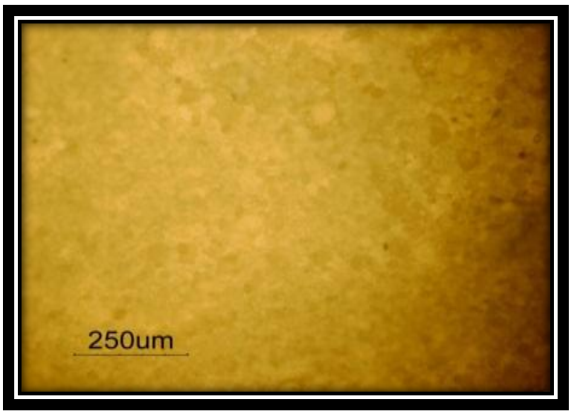


**Figure (7):** Microphotograph showing bone tissue around a nano-hydroxyapatite (HA) coated implant after 2 weeks, stained with H&E, magnification x20.

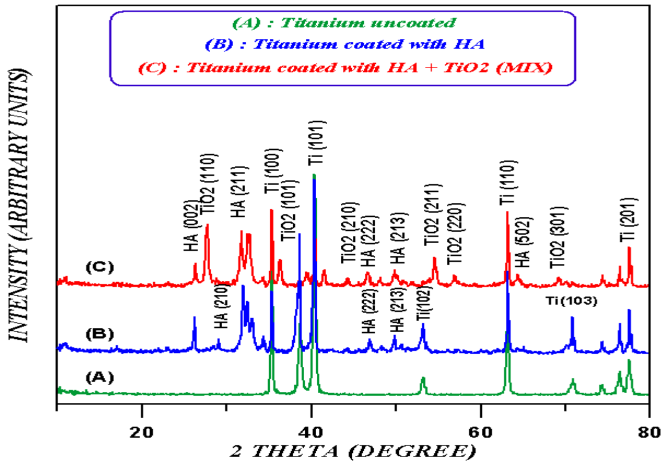


**Figure (8):** X-ray diffraction patterns were recorded for (A) the uncoated sample, (B) the specimen coated with nano-hydroxyapatite (HA), and (C) the sample coated with a mixture of nano-HA and nano-$TiO_2$ on commercially pure titanium discs.

**X-ray Diffraction of nano coated disc**: As shown in Figure (8), the dip-coated surface is uniformly covered with hydroxyapatite (HA) and a combination of HA and $TiO_2$. This is confirmed by the XRD pattern, where most diffraction peaks correspond to the crystalline phases of HA and $TiO_2$, in accordance with the standard JCPDS files: #9-432 for HA, #44-1294 for titanium, and #21-1276 for $TiO_2$.

**Histological test:**

**1) Two weeks after implantation**

**A-**

A histological examination of the nano-HA coated implants revealed visible implant threads (indicated by arrows), along with newly formed delicate collagen fibers and osteoid tissue (OST) developing within the bone marrow spaces, as shown in Figure (9). In contrast, the bone tissue surrounding Cp Ti implants coated with a nano-HA and nano-$TiO_2$ mixture demonstrated the presence of bone trabeculae (arrow) occupying the thread apex, with observable bone marrow (BM) components, as illustrated in Figure (10). Additionally, osteoclasts (arrow) were noted occupying Howship's lacunae at the base of the implant site, adjacent to regions of woven bone (WB), as seen in Figure (11).

**Figure (9):** Microphotograph showing bone tissue around a nano-hydroxyapatite (HA) coated implant after 2 weeks, stained with H&E, magnification x20.

**B-**

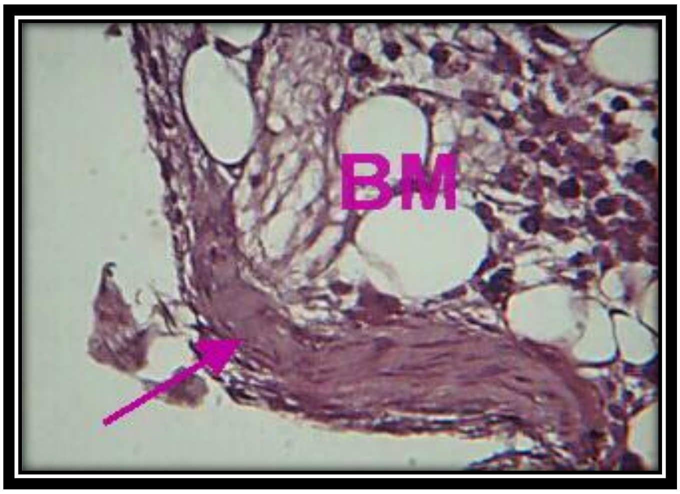


**Figure (10):** Bone trabeculae (arrow) occupies apex of the thread, bone marrow (BM), H&E×40

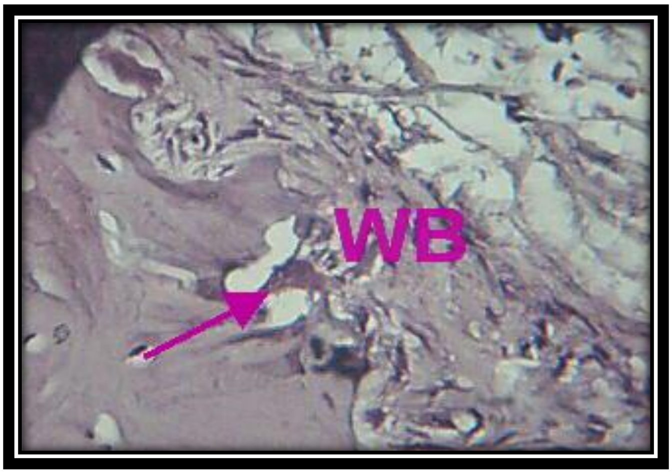


**Figure (11):** Shows an osteoclast (indicated by an arrow) residing within a Howship's lacuna at the base of the implant bed, in close proximity to woven bone (WB), as observed under H&E staining at 20x magnification.

**2) Six weeks after implantation**

the histological analysis of implants coated with nano-hydroxyapatite (nano HA) reveals visible implant threads (arrows), along with the formation of woven bone (WB) and bone trabeculae (BT), as illustrated in Figure (12). Another microscopic image, Figure (13), demonstrates trabecular bone (BT), osteocytes (arrows), and osteoblasts (arrowheads) present along the bone surface. In Figure (14), the section of commercially pure titanium (Cp Ti) implants coated with a combination of nano-hydroxyapatite (HA) and nano-titanium dioxide ($TiO_2$) reveals new bone (NB) formation merging with the existing basal bone (BB) after six weeks of healing, Figure (15) displays mature trabecular bone, with osteoblasts (arrows) lining the bone surface and osteocytes (arrowheads) embedded within the bone matrix.

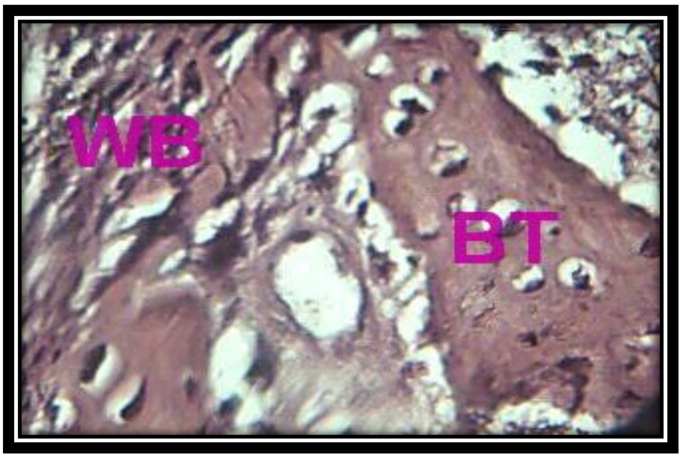


**Figure (12):** Displays woven bone (WB) deposition and trabecular bone (BT) formation, along with visible implant thread impressions (arrows), stained with H&E, magnification ×40.


**Figure (13):** Bone trabeculae (BT) coalesce with basal bone(BB). H&E×10

**B-**

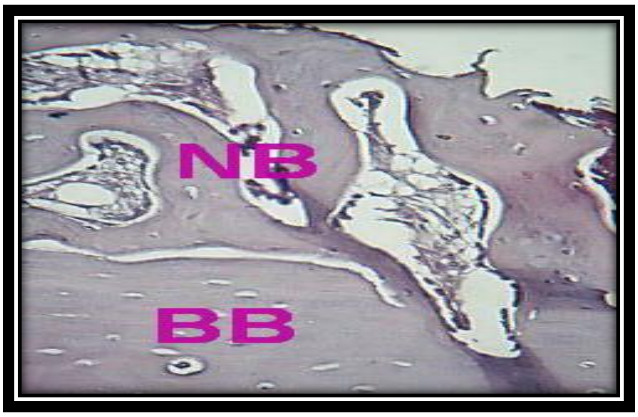


**Figure (14):** Microscopic examination reveals newly formed bone (NB) merging seamlessly with the underlying basal bone (BB). Stained with H&E, magnification ×20.

**Figure (15):** Mature trabecular bone is evident, with osteoblasts (arrows) lining the bone surface and osteocytes (arrowheads) embedded within the bone matrix. H&E stain, magnification ×40.

**Discussion**

Improving the integration of metallic implants with surrounding bone tissue can be achieved through the application of a thin biocompatible ceramic coating. One effective method for achieving a uniform and pure coating on the implant surface is dip-coating (11, 19,20,21)

Optical microscopy of coated implants prior to insertion showed no signs of cracking in the ceramic layers produced using the dip-coating technique for both types of coatings. This absence of cracks may be attributed to the controlled deposition process, low-temperature sintering, and precise adjustment of the $P_2O$ content (0.01 g in 50 ml ethanol). This observation contrasts with the findings of (19), who reported visible cracks in the coating under optical microscopy.

X-ray diffraction (XRD) analysis confirmed that the surface of the coated implants was thoroughly covered with a nanolayer consisting of hydroxyapatite (HA) and a HA–$TiO_2$ mixture, as evidenced by the characteristic diffraction peaks corresponding to both HA and $TiO_2$. These results are consistent with previous studies demonstrating that X-rays can penetrate through thin ceramic layers to identify their structural composition [27] .

Histological examination of implants coated with nano HA after two weeks of insertion in tibial bone revealed early formation of an osteoid (woven bone) layer near the implant surface, along with isolated bone islands within the marrow. These observations support previous findings [23,24,25] , which described early stages of new bone development, even when different implant designs, materials, and techniques were used.

Similarly, implants coated with a nano mixture of HA and $TiO_2$ exhibited extensive trabecular bone development with abundant neovascularization after two weeks. Osteoblasts were seen lining the trabeculae, indicating active bone formation. These findings suggest that HA–$TiO_2$-coated implants promote robust early-stage osseointegration, making them suitable candidates for immediate implant loading. This outcome aligns with reports from [10, 26], which documented increased bone growth around bioceramic-coated titanium implants within a two-week period.

After six weeks of implant placement, implants coated with nano-hydroxyapatite (HA) and titanium dioxide ($TiO_2$) showed new bone integrating with the basal bone, along with the presence of mature, trabeculated bone. Osteoblasts were observed lining the implant surface, and osteocytes were embedded within the bone matrix. These findings are consistent with previous studies [20, 21, 23] , Similar patterns of bone formation were observed within the threaded areas of implants coated with nano-hydroxyapatite (nanoHA) and titanium dioxide ($TiO_2$), even after comparable healing periods.

The findings clearly demonstrate that bone tissue developed around all coated implants, with no evidence of inflammation or fibrous tissue encapsulation, regardless of the coating composition or healing time. This supports the idea that the healing outcome is strongly influenced by the implant's surface characteristics. These findings align with previous research[20, 21], which concluded that bioactive coatings enhance bone regeneration.

## Conclusion:

The results showed early bone formation, mineralization and maturation aroung Ti screws coated with mixture of nano HA & nano $TiO_2$ in comparison to Ti screws coated with nano HA.

## Acknowledgements:

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## Conflicts of interest:

The authors declare that they have no conflicts of interest.

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