Evaluation of Mechanical and Histological Significance of Nano Hydroxyapatite and Nano Zirconium Oxide Coating On the Osseointegration of CP Ti Implants

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ABSTRACT

Background: Dental implant considers a unique treatment option for the replacement of missing dentition. The new trend of implants is looking for materials which accelerate bone formation in bone implant interface and enhance osseointegration to provide immediate loading directly after placement and decrease the time period which is disturbs patients and uncomfortable. The aim of the study was to evaluate the effect of nano zirconium oxide (ZrO_2) and nano hydroxyapatite (Hap) mixture coating of screw shaped commercially pure titanium (cpTi) implants on bond strength at the bone implant interface with torque removal test and histological analysis in comparison with non coated implants.

Materials and methods: Forty screws were machined from cpTi rods using a lathe machine. Then 20 screws coated by electrophoretic deposition method by a mixture of nano hydroxyl apatite and nano zirconium oxide, while the other 20 screws remain uncoated. The tibia of 10 adult white New Zealand experimental rabbits was chosen as implantation site, each rabbit tibia received 2 screws, one coated and the other uncoated. Torque removal test was performed to measure the torque required to remove the screw and histological analysis was performed to observe the new bone formation, after 2 and 4 weeks healing intervals.

Results: Implant coated with a mixture of nano zirconium oxide and nano hydroxyl apatite showed a significantly higher removal torque values compared to uncoated one. There was more new bone formation with coated implants for both healing periods.

Conclusions: Coating by electrophoresis considers a valuable process to coat metallic implants with a ceramic material and to form a uniform composite layer of coating. Osseointegration improved at bone-implant interface associated with the coated implants, which was illustrated by higher bone formation at the two intervals of time 2 weeks and 4 weeks.

Keywords: Implant materials, Bone-implant interface, Nano surface coating. (J Bagh Coll Dentistry 2016; 28(3):30-37).

INTRODUCTION

Bone can be morphologically classified to cortical and cancellous. The cortical bone structure called compact and it responsible for mechanical support. Cancellous bone is responsible for the bone metabolic functions, it surrounded by compact bones [1].

The ultimate goal of using implant is the prosthetic rehabilitation of patient with missing dentition for both the functional and esthetic level [2].

Generally, dental implants can be classified into endosseous implants, subperiosteal implants and transosseous implants [3].

Dental implants differ in a lot of aspects like implant material, implant dimensions, surface properties of implants and interface geometry [4].

It is important to note that osseointegration defined as the apparent direct attachment or connection of osseous tissue to an inert, alloplastic material without intervening connective tissue [5]. In practice its mean that in osseointegration there was an anchorage mechanism that persists under all normal loading conditions in which non vital components can be incorporated into living bone tissue [6].

Furthermore osseointegration never occurs on 100% of the implant surface. Successful cases will have range of osseointegration between 30% and 95% of dental implant surface is contact with bone tissue, as measured by light microscope [7].

The technology of ceramic materials in dental applications was steadily introduced. The improvement in ceramic toughness and strength make it possible to increase the range of dental applications to dental implants and abutments [8].

It has been shown that preparation techniques of titanium implant significantly affect the surface properties and also the biologic response of bone tissue that occur at the surface [9].

Electrophoretic process used during coating of ceramic particles on titanium substrates are of particular interest because they increase the bone healing around titanium implant. Electrophoretic deposition result in homogenous deposit layer and provide easy controlling on the thickness of coated layer over a different structures [10].

The purpose of this study was to evaluate the effect of mixture from nano zirconium oxide and nano hydroxy apatite as ceramic coating materials on the strength of bone-implant interface after implantation in rabbit tibia bone by means of torque test and histological analysis.

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MATERIALS AND METHODS

Sample preparation

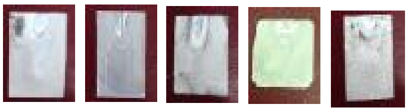
The substrate of coating used in the study was from titanium plate, which was cut by sharp scissor from titanium sheet to square shape specimens of (15 × 15 ×0.25mm) width, length and thickness.

These Ti specimens have a polished mirror surface placed in ultrasonic bath of ethanol in order to get rid of contamination and debris in 15 minutes, then for 10 minutes in distilled water bath, after that the specimens left to dry at room temperature. The platescoated with a mixture of nano zirconium oxide and nano hydroxyapatite for 3 minutes and 60 volts according to the pilot study results.

Pilot study

In pilot study titanium plates coated with a mixture of nano zirconium oxide and nano hydroxy apatite under 60V for 1, 3, 5, 7 and 9 min [(11)].

The plates placed in a beaker containing a mixture ofcoating particles, the anode used for particles charging. The Ti specimen was attached to the cathode and a Ti sheet with the same size and dimension was attached to the anode.After electrophoretic coating theplates sintered at 820°C for densification to be ready for testing by optical microscope as shown infigure 1. [(12)]



1min. 3min. 5min. 7min. 9min.

Figure 1:Ti plate after coating

Examination of surfaces:

a. Microscopical examination

The optical microscope (Nikon Type 120, Japan optical microscope) with a digital camera type DXM 1200 F, used to examine the appearance of each sample of coating. From this examination specimen coated at 3min. seen with homogenous smooth surface, this specimen further analyzed by x ray diffractometer and atomic force microscope tests.

b. X-Ray phase analysis

Phase analysis was employed on the sample after coating at 3min and 60V using Shimadzu Lab XRD - 6000 Powder X ray diffractometer using Cu Kα radiation.

c. Surface roughness measurement

Ti plates coated at 3min and 60V were examined for surface roughness using scanning probe microscope which give information about the average roughness of the coating layer.

Implants preparation

Forty screws were machined from commercial pure titanium rods using a lathe machine with cutting head coated with titanium carbide the length of the screw was 8mm (5mm was threaded and 3mm was flat) and the diameter was 3mm.They have a slit in the head of 1.5mm depth and 1mmwidth to fit the screw driver and torque meter. These screws washed in ethanol in an ultrasonic cleaner for 15 minutes to remove the debris and contamination and then dried at room temperature as shown in figure 2.

Figure 2: Screws shaped implants

Out of the 40 screws, 20 screws were left as machined implants and the remaining 20 screws were coated by electrophoretic deposition process with a mixture of nano zirconium oxide and nano hydroxyapatite (3min., 60V). Coated screws sintered by using carbolite furnace for 820 ºC under inert argon gas to prevent oxidation. Then the screws were sterilized with gamma radiation and kept in airtight sheets till the operation day.

Surgical procedure

Ten adult white New Zealand experimental rabbits were usedtheir weight range between 1.5 to2 Kg. The age of the animals was from 10-12 months. They were left for 14 days in the same environment before surgical operation.

Subcutaneous one dose of 10 mg ivermectin injection was given to ensure parasite free animals. Each animal was weighed before operation to determine the required dose of anesthesia and antibiotic. The animals were anaesthetized with a combination of ketamine (25mg/kg) and xylazine (17.5mg/kg) intramuscularly.

The legs were shaved by shaving spray, washed and decontaminated with a mixture of iodine and 70% ethanol. The tibia metaphysics was exposed by incision through skin, fascia and periosteum then the skin and fascia flap was reflected to expose the bone. Bone penetration was performed with a round guide drill to make two holes with 10 mm distance between them, continuous normal saline cooling associated with intermitted pressure at a rotary speed of 1500 rpm and reduction ratio 16:1 were used during surgery. The implant bed enlargement obtained gradually with spiral drills until size 2.8 mm.

The coated screw was removed from air tight sheet, placed in the first hole (proximal one) using small screw driver which fit the implant slit until the first 5 mm of titanium implant totally inserted inside the tibia bone. The non coated screw placed in the second hole (distal one). The final screwing was done with torque meter (approximately 10 N.cm). Then catgut suture 3/0 used for suturing the rabbit muscle followed by using silk suture 3/0 for skin suturing, local antibiotic applied over the operation site, followed by systemic antibiotic injection.

The operation site was radiographed three times using conventional x-ray machine and per apical technique. The first one was taken prior to the surgery to ensure sufficient bone for implants and the second x-ray was taken immediately after surgery to investigate a proper position of implants, finally the third x-ray was taken before sacrificing the animal to evaluate the effect of these implants on the surrounding bone.

Mechanical test

The same surgical instruments and anesthetic solution used in the implantation procedure. Four animals were used for mechanical testing from each healing period 2 and 4 weeks using torque meter. The rabbits were anesthetized with the same dose used in the surgical phase. Incision was made at the lateral tibia side then muscles and fascia were reflected and removed to expose the entire animal bone. A torque removal test applied by engaging the screw driver of the torque meter into the implant slit to measure the maximum torque necessary to unscrew the titanium implant.

Histological test

For each healing interval 2 and 4 weeks one animal were used for histological test with optical microscope. The rabbit was injected with an overdose of anesthetic solution. Cutting of the bone around the implant was performed using a disc in low rotating speed handpiece with normal saline cooling.

Bone-implant blocks were immediately stored in 10% freshly prepared formalin and left for 3 days for fixation. After fixation, the specimens were then left in a solution of sodium citrate and 10% formic acid in order to decalcify the bone.

After 2-3 weeks the specimen was checked for complete decalcification by penetration of a narrow needle to the deepest part of the bone implant block,when decalcification complete the bone- implant block was divided into two parts using a sharp scalpel one of them containing the implant, then the implant was gently removed from its bony bed. [13]

Bone tissue was gradually dehydrated by being passed through a series of increasing percentage of alcohol (70%, 80%, 90% and absolute alcohol) remaining in each one for 1 hour. Specimens then passed through two changes of xylene for 15-20 min and placed in a dish of melted paraffin. The dish was placed into a constant-temperature oven regulated to about 60˚C. Finally, the specimens were molded in the center of paraffin block, andadjusted to a microtome where serial sections of 5-µm thickness for each part of the bone was performed one of every 10 sections was taken and placed on a slide.

The slide was placed in a container having haematoxylin and eosin stain for 10 minutes to stain the tissue,then it was removed from the staining container, rinsed with deionized water and a glass cover was fixed on the stained tissue with canada balsam.Photograph of each section taken by light microscope and SAMSUNG camera at x4 power magnification.

RESULTS

Optical microscopical observations

A series of micrographs illustrate the coating titanium surfaces for different times and 60 voltages. There is a homogeneous thickness layer over the titanium substrate during coating with 60V and 3 min. as shown in figure 3.

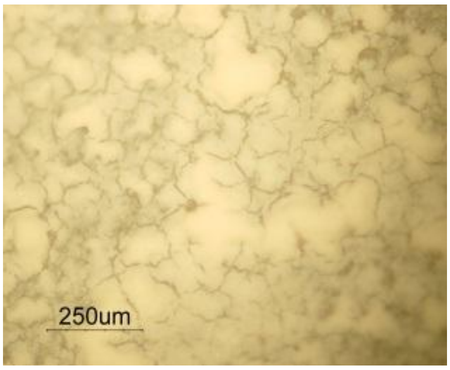


Figure 3: Optical micrograph view of cpTi coated with mixture of nano Hap and nano ZrO_2 for 3min. and 60V (100x)

X-ray diffraction of coating sample

After electrophoretic deposition, it is evident from figure 4 that the titanium specimen was covered by a mixture of nano zirconium oxide and nano hydroxyapatite surface because there was diffraction peaks could be indexed to zirconium oxide and hydroxyapatite phases.

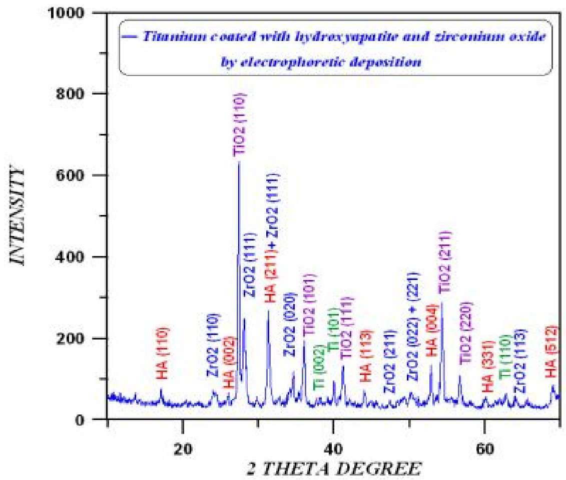


Figure 4: X-ray diffraction patterns of nano ZrO_2 and nano Hapcoated cpTi specimen

Atomic force microscope of coating sample

Coated specimen appear with small homogenous embedded nodules with average roughness 1.95 nm which are important for the osseointegration as illustrated in figure 5.[14]

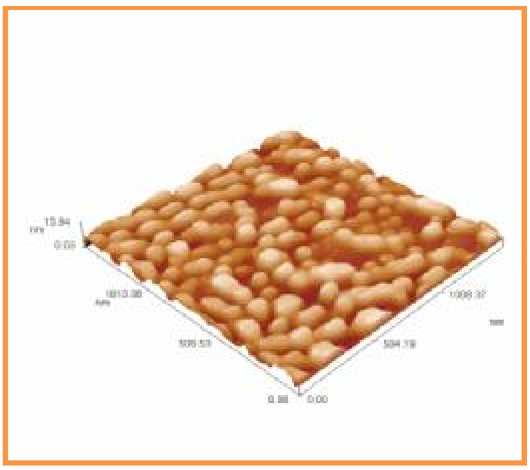


Figure 5: AFM topographies of the coated surface

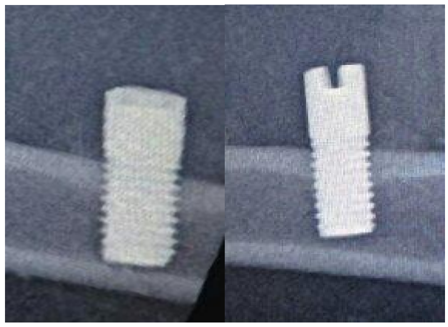
In vivo experiments:

<u>Clinical observations</u>

All experimental rabbits tolerated the surgical procedure and moved normally within one weekafter operation. There was no any sign of tissue reaction or bone infection seen around the overall screw bed in any rabbit. All implants were found stable in the bone, they could not be moved with manual force and there were no defect coronally around any implant after 2 and 4 weeks of healing periods.

<u>Radiological observations</u>

The results of radiographic evaluation showed that there was no radiolucent zone between the cortical bone tissue and the implanted screw with all examined screws as shown in figure 6.



Coated Non coated

Figure 6: Radiograph of cpTi implants after 4 weeks of implantation

<u>Mechanical test</u>

Descriptive statistics of removal torque values of uncoated implant and coated implant by electrophoretic deposition after 2 and 4 weeks of implantation are shown in table 1. The higher torque value was needed to remove the coated screws with mean value of 13.411N.cm and the lowest torque value was needed to remove the non coated screws with mean value of 8.999N.cm in the 2 weeks interval.

Also in the 4 weeks interval the higher torque value was needed to remove the coated screws with mean value of 20.033N.cm and the lowest torque value was needed to remove the non coated screws with mean value of 14.295N.cm as shown in figure 7.

Table 1: Comparison of mean of torque value of coated and non coated group between 2 and 4 weeks intervals

State	Duration	N	Mean	S.D.	S.E.	Min.	Max.
Non-coated	2 weeks	8	8.999	1.052	0.372	7.76	10.59
	4 weeks	8	14.295	2.288	0.809	11.29	17.65
Coated	2 weeks	8	13.411	1.811	0.640	11.29	15.53
	4 weeks	8	20.033	2.294	0.811	16.94	23.3

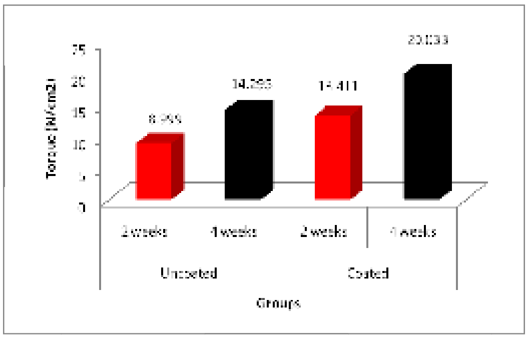


Figure 7: The differences in the torque mean values between all groups

t – test of the equality of torque values means between uncoated and coated screws at 2 weeks healing period showed a highly significant difference, also at 4 weeks as demonstrated in table 2, 3.

Table 2: t-test for coated and uncoated implants at 2 weeks interval

Types	Mean Difference	t-test for Equality of Means			
		t	df	p-value	Sig.
Non coated × Coated implants	-4.413	-5.960	14	0.000	HS

Table 3: t-test for coated and uncoated implants at 4 weeks interval

Types	Mean Difference	t-test for Equality of Means			
		t	df	p-value	Sig.
Non coated × Coated implants	-5.738	-5.01	14	0.000	HS

Histological features

The histological view of non coated titanium implants after two weeks of implantation illustrate apposition of osteoid tissue of implantimpression without detection of distinct threads as shown in figure 8.

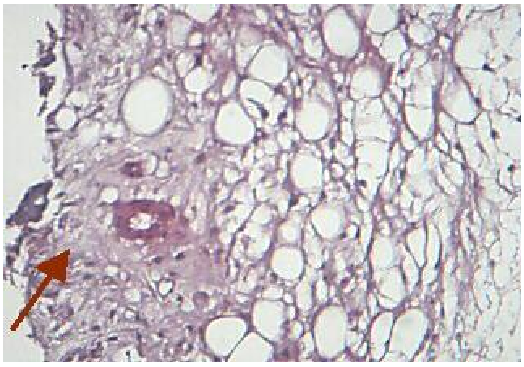


Figure 8: Microphotograph view for control group at 2 weeks duration shows apposition of osteoid tissue (arrow)

Histological findings of implanted screws coated with a mixture of nano zirconium oxide and nano hydroxyl apatite after two weeks of implantation illustrate thread of implant impression and osteoid tissue occupies apex of the thread. Also bone trabeculae occupies base of implant bed close to basal bone, osteoblast rimming bone and osteocyte can be detected as shown in figure9.

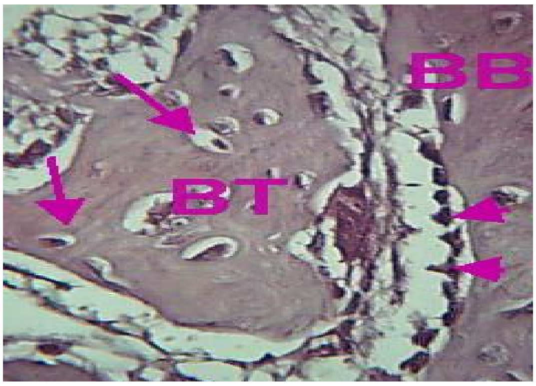


Figure 9: View for bone trabeculae (BT) occupies base of implant bed close to basal bone (BB), osteoblast (arrow heads) rimming bone and osteocyte (arrows) can be detected. H&E×20

The histological view of non coated titanium implants after four weeks of implantation illustrates threads of implant impression. Immature bone can be detected at the base of the impression bed and osteocytes scattered randomly within bone matrix as shown in figure 10.

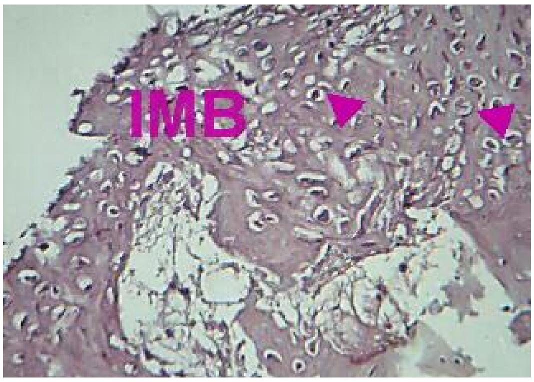


Figure 10: Immature bone (IMB) at the base of the impression bed, osteocytes (arrow heads) scattered randomly within bone matrix.H&E×20

The coated implants at 4 weeks show interdigitation of opposing threads of implant impression and the bone surface surrounded by osteoblast. The base of implant impression close to basal bone filled by bone trabeculae and the osteocyte easily detected as shown in figure 11.

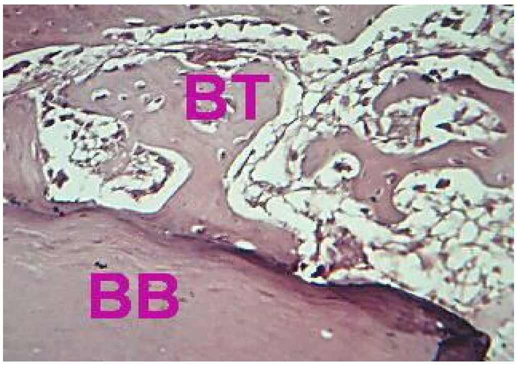


Figure 11: Bone trabeculae (BT) coalesce with basal bone (BB). H&E×10

DISCUSSION

In vitro experiments

<u>The electrophoretic deposition</u>

Commercially pure titanium was satisfactorily coated with a mixture of nano zirconium oxide and nano hydroxyapatite. The electrophoretic deposition of biocompatible ceramic materials on titanium substrates considered as a step in the way of implants improvement because of the adhesion between the ceramic coating and the titanium substrate and therefore increase the implant-bone osseointegration and the lifetime of dental implant in the living tissue.[15]

The electrophoretic deposition method shows a continuous thin, uniform thickness coating layer, this method form a homogenous coating thickness on the titanium implant independent on the shape or surface as illustrated by **Zhitomirsky** [11].

The study agreed with **Bersa and Liu** who confirmed the using of nanoscale particle size has a great influence on crack control of the deposit layer [16].

<u>XRD phase analysis</u>

It is obvious from the figure of the XRD (Figure 4) that the specimen surface covered with the ceramic mixture because there were diffraction peaks could be indexed to zirconium oxide and hydroxyapatite phases. These phases are a stable phases at room temperature as confirmed by **Zhang et al**. [17].

The presence of Ti peaks in the XRD pattern after coating process is due to the penetration of x-rays beyond the coating layer.

<u>Atomic force microscope (AFM)</u>

The coating method used in this study gives nano roughness which appeared from the grain size that was in nano diameter. This is also supported by the results of optical microscope. After deposition of coated layer the surface roughness increased which can be estimated from the peaks that appeared on the surface and also the diameter of grains.

Results measurement provided from the scanning probe microscope revealed that the coating method increases the roughness of the surface lead to increasing surface area on the implant and promote the opposition of bone which agrees with **Albrektsson and Wennerberg** [13].

In vivo experiments

<u>Implant preparation prior to surgery</u>

The screw type considered the most implant designs used in rabbit models. Regardless of screw design, it should have an appropriate size for the bone tissue site and for the animal used.

The key for implants clinical success is the primary stability in bone. It is depend on bone tissue density, the implant design and the technique of surgery.[18]

The implant stable in the tibia bone after implantation and this can attributed to the screw type implant used and also to a thick cortical bone and this agreed with **Pearce et al.** who found that the screw type implants have the advantage of producing good initial stability [19].

While **Miyamoto et al.** consider the initial stability at the implant insertion time is influenced more by cortical bone thickness than by implant length. [20]

Experimental animal description

Rabbits used as a study model having many advantages include the ease of manipulation, rapid bone healing response compared to other models, also the metabolic activity of animal bone tissue similar to that of one third of human. [21]

The age of rabbits used was about 10 months as a minimum age to ensure growth stop of the proximal rabbit tibia and more similar to bone physiology of adult human bone tissue. [22]

The tibiae sites in the rabbit were chosen to mimic the clinical situation and since the dimensions of this bone correspond well with human alveolar space. Also this tibia bone model has low morbidity and easy access by titanium implant, **Dahlin et al.** stated that the rabbit tibia bone morphology allow the titanium screw to engage bone cortex at coronal area and marrow apically [23].

In this study the size of the holes created in the bone was smaller than the diameter of the implant which results in a surgical fit. The holes were made 2.8 mm while the implants were 3 mm diameter, this agrees with **Skalak and Zhao** [24].

Radiographical examination

The radiographic examination in this study demonstrated a bone to implant direct contact, there was no radiolucent zones or any abnormal reaction to the implant. However, the absent of radiolucent area is not evidence for implant-bone osseointegration, since it is impractical for a person to diagnose bone loss by x-ray at 0.1 mm resolution and the soft tissue cell size in the range of 0.01 mm for that a very narrow fibrous tissue zone undetectable by x-ray as illustrated by **Atsumi et al.** [25].

Mechanical test

Removal torque was used in this study as an indicator for the presence of osseointegration. Torque defined as the movement applied by twisting force on the body at a distance that was equal to the perpendicular distance between the force action line and the rotation center multiplied by the force magnitude.[26]

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 [27,28].

Effect of time on removal torque value

The results illustrate that there was an increase in the removal torque value with time which may be due to progressive bone formation in bone-metal contact with time and remodeling around the implant during healing period that consequently improve the mechanical capacity, and this agreed with a removable torque studies in rabbit carried out by **Cho and Jung** [29].

Effect of coating surface on removal torque value

The porous surface of the coating layer stimulated bone formation more than non coated surface. This finding was confirmed by **Suzuki et al.** who reveal that porous particles in the coating layer can directly adhere to bone tissue in vivo that induce early bone ingrowth [30].

Clokie and Bell found that the more cortical bone tissue which contacts dental implant required higher removal forces, and the surface of the coated implant increase bone to implant contact [31].

In conclusion; the rabbits can normally tolerate the coating materials and the implants and that illustrated by the absent of infection. Higher torque removal mean values for the coated implants compared to non coated one at two implantation periods andthis values increased with time for both coating and non coating screws.

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