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The Effect of Implant Screw Coating with Nano-Hydroxyapatite and Magnesium Chloride Mixture on Osseointegration: Biomechanical and Histological Study

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ABSTRACT

The goal of modern implantology is fine and fast osseointegration which is a major factor influencing the success of dental implantation, and its largely depend on the implant surface. High-quality osseointegration stand for an accelerated healing process, high stability, and durability of the dental implant. ***Aim:*** *To evaluate the effect of composite material coating which includes ceramic nano hydroxyapatite and magnesium chloride on the bond strength at bone implant interface and tissue reaction.* ***Methods:*** *In in vitro study, a plate of Cp-Ti was coated with hydroxyapatite and $MgCl_2$ by electrophoretic deposition coating technique. Coating procedure was performed using different proportion of hydroxyapatite and $MgCl_2$ and different coating time at fixed voltage. Then analysis of coated surface was performed. The tibia of white male New Zealand rabbits were chosen as implantation sites of 40 screws for in vivo study. Torque removal test was performed to measure bond strength between implant and bone after 2 and 4 weeks healing periods.* ***Results:*** *The results of Energy-dispersive X-ray spectroscopy shows that 1.9% of Mg and 32.4% Ca contain on Ti substrate. Optical microscope examination showed that 2 minutes is the suitable time used for coating screws at 30 V. Analysis of surface roughness in nanoscale was 86.89 nm and grain size 128 nm while scanning electron microscope showed numerous agglomerations of small spherical particles between 5 nm and 100 nm of hydroxyapatite. The torque mean value at bone-implant interface in coated implants was significantly higher than the uncoated implants at 2^{nd} and 4^{th} week. In histological analysis, there was an interdigitating of well-developed bone close opposing threads of coated implants after 2 and 4 weeks implantation. Coating Cp-Ti screws with mixture of hydroxyapatite and $MgCl_2$ was efficient in increasing bone bonding strength to dental implant at bone implant interface than uncoated implant, which was demonstrated by higher torque removal force and increase in bone formation around screw with time.*

Keywords: Electrophoretic deposition, Magnesium chloride, Screw, Dental implant, Torque, Hydroxyapatite

Abbreviations: Cp-Ti: Commercially-Pure Titanium; HA: Hydroxyapatite; XRD: X-ray Diffraction; AFM: Atomic Force Microscope; SEM: Scanning Electron Microscope; EDX: Energy Dispersive X-ray Spectroscopy; EPD: Electrophoretic Deposition

INTRODUCTION

Dental implant is an important therapeutic approach, providing both an aesthetic and functional alternative to tooth replacement [1]. Osseointegration is the close contact between bone and implant [2]. Long term success of dental implant largely depends on rapid healing with safe integration into the jaw bone [3].

Bone/implant interface is complex and involve numerous factors. Implant surface probably have the greatest potential for enhancement in implant dentistry. Characteristics, such as surface composition, surface topography and surface roughness affect the mechanical stability of the implant tissue interface [4].

Surfaces of bone implants represent the site of interaction with the surrounding living tissue and are therefore crucial

to enhance the biological performance of implants [5,6]. Deposition technique is more efficient techniques and can be used for deposition of nano size particles on complex shape components. Also, this method can be easily done, versatile and low cost [7].

The wide using of EPD method attracted much of interest, that's can be deposition with any size of particle in powder from such as oxides, metals, polymers, carbides, and nitrides [8].

The most important function of a coating in this context is to modify the surface of an implant to improve fixation to the surrounding tissue [9].

As a desirable bone replacement material, hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, with stoichiometric composition, it has drawn a great interest for use in the repair of bone defects and in coating on metal parts of prosthetic implants due to its excellent biocompatibility, bioactivity, and bone-bonding properties [10].

The advantages of using HA nanoparticles may be offset by the fact that nanoparticles may also be more reactive than micro particles and could lead to undesirable reactions with the underlying metallic substrates at lower temperature than conventional (micrometric) particles, thus resulting in unstable HA/substrate interfaces [11,12].

The biological influence of nano rough surfaces is a relatively new area of research and highly interesting since several studies have indicated that nano-topography can enhance osseointegration. When combining the two surface entities, nano roughness and bone-like chemistry, for example, by using nan sized hydroxyapatite, a synergistic effect can be generated [13].

Magnesium has been shown to have beneficial effect when incorporated locally at certain doses in bone by stimulating bone formation [14].

Magnesium and its alloys are metallic biomaterials that can be biodegradable in the body fluids and also it is an essential element for bone metabolism and may promote the formation of new bone tissue [15].

Orthopaedic applications for magnesium materials have been shown to achieve enhanced bone response and excellent interfacial strength [16]. Also, magnesium is used for different types of fixation devices in orthopaedic surgery, such as screws, plates, and fasteners [17,18].

Magnesium-incorporated micro/nanostructured titanium surface are potential candidates for clinical applications to improve bone and titanium integration [19]. Due to the unique characteristic of dissolving readily in an aqueous solution, in recent years, there has been significant increase in the research on magnesium and magnesium-based alloy into a development of new biodegradable orthopaedic material [20].

In addition, magnesium and magnesium-alloys support the development of stromal cells towards osteoblasts and subsequently stimulate the production of extracellular matrix [21].

The present study, using nano hydroxyapatite mix with $MgCl_2$ as a coating biocompatible material on Cp-Ti implant screw for improving the functional and biological efficacy of titanium implant.

MATERIALS AND METHODS

In vitro study

EPD coating procedure

EPD coating procedure started with suspension preparation and the main component of this suspension are ethanol absolute ≥ 99.8% as a solvent, Iodine as a dispersant and phosphoric acid as a binder [22]. Two types of pilot studies were done for selection of the suitable suspension with proper concentration of HA/$MgCl_2$ and suitable voltage.

In the first pilot study, suspension consists of (40%) 2 gm/L (ethanol) nano HA powder, (20%) 1 gm/L (ethanol) $MgCl_2$ powder and 0.5 gm iodine, then these powders added to the solvent which was the 50-ml ethanol absolute ≥ 99.8% in a container over a stirrer. The stirring at normal speed was continued until a colloidal suspension was obtained at room temperature. Then 2 drops of phosphate ester as dispersant agent was added to the suspension before coating. The voltage applied during coating 60 V for 1/2 min. The result of coating unsuccessful, irregular coating with poor adhesion and XRD was unclear

In the second pilot study, suspension consists of (80%) 4 gm/L (ethanol) nano HA powder, (20%) 1 gm/L (ethanol) $MgCl_2$ powder and 0.5 gm iodine, then these powders added to the solvent which was the 50-ml ethanol absolute ≥ 99.8% in a container over a stirrer. The stirring at normal speed was continued until a colloidal suspension was obtained at room temperature. Then 2 drops of phosphate ester as dispersant agent was added to the suspension before coating. The voltage applied during coating 30 volts for at different time periods (0.5 min, 1 min, 2 min, 3 min). Analysis of coated surface was done by:

Elemental analysis (X-Ray Diffraction)

Phase analysis was studied using Cu K α radiated X-ray diffraction method which was done for coating sample for characterization and identification of polycrystalline phases that extracts detailed information about the chemical composition and crystallographic structure of natural or manufactured materials. The 2θ angles were swept from 20°-60° in step of one degree. The peak indexing was carried out based on the JCPDS (Joint Committee on Powder Diffraction Standards).

Structural surface characterization

An optical microscope (Nikon Eclipse ME600L/441002, Japan) provided with digital camera type DXM 1200 F. Nikon ACT Version 2.62, 2000 software was used for examination of the surface feature of coated layer. Scanning electron microscopy (SEM Test speed Vega 111, USA) used for examination of the surface morphology and material characterization.

Examination of the surface morphology in nano scale includes an electron beam scanned over the sample surface. The electron beam induced a larger depth of focus than a regular light beam and images at very high resolution can be recorded.

Material characterization was done by using EDX analysis. This was performed within the SEM instrumentation. When the incoming electron beam interacts with the sample, this can cause emission of X-ray photons due to the excitation and relaxation of sample atoms, Since the emitted X-ray photons are characteristic for each element, EDX is used for both qualitative and quantitative elemental analysis [23].

Preparation of screws

Forty screws shaped implants, 3.0 mm in diameter and 8 mm in length (threaded part is 5 mm and smooth part is 3 mm) and pitch height is 1 mm were machined from Cp-Ti rods Grade 2, with slit in head of the implant to fit the screw driver during insertion and removal by torque meter. The screws were divided into two groups each group consisted of twenty screws. The first group of screws was coated with mixture of HA and $MgCl_2$ for 2 min with 30 volts following the same procedure of EPD of second pilot study that was mentioned previously. While the second group of screw was uncoated.

Design of study

The screws were categorized according to the test performed into:

1. **Mechanical (torque measurements) group:** (32 screws). The screws were divided into:
   a) **Control group (16 screws):** This group includes 8 screws for each healing interval (2 and 4 weeks).
   b) **Experimental group (16 screws):** This group includes 8 screws for each healing interval (2 and 4 weeks) coated with mixture ($HA/MgCl_2$).
2. **Histological test group: (8 screws)**: In this test the screws were divided into:
   a) **Control group (4 screws)**: This group includes 2 screws for each healing interval (2 and 4 weeks).
   b) **Experimental group (4 screws):** This group includes 2 screws for each healing interval (2 and 4 weeks) coated with ($HA/MgCl_2$).

***In vivo* study**

Ten adult male New Zealand White healthy rabbits weighting 2-3 kg were used. The age of animals was from 10-12 months. Animals were left for 14 days in the same environment before surgical operation. They had free access to tap water, and were fed with standard pellets, jet and carrot. The total animals were divided in to two groups for each

healing interval (2 and 4 weeks) each one consists of 5 animals, 1 of them were sacrificed for histological study, while the other 4 were sacrificed for mechanical test for torque removal test. All implants (2 uncoated and 2 coated) were implanted in both tibia, (each tibia received one implant uncoated and one coated) consequently starting from the medial to the distal metaphysic for each animal.

For screw implantation in tibia of rabbit, surgical procedure was performed according to Helsinki in 1964 [24] Coated screw was placed in the first hole (proximal one) using screw driver first then torque meter was used, so 5 mm length of screw introduced in bone completely, then uncoated screw holds to second hole (distal one), then suturing of muscles was done with absorbable catgut suture 3/0 followed by skin suturing with silk suture 3/0, postoperatively care was performed by giving local and systemic antibiotic (20 ml/kg procaine and penicillin) for 5 days after surgery.

Mechanical test (Torque test)

For each healing interval (2 and 4 weeks) eight animals were used for mechanical testing by removal torque. The animals were anesthetized with the same type and dose that used in the implantation procedure.

The entire tibia was exposed and supported firmly while performing mechanical test to prevent any movement which may affect the accuracy of the test.

A torque removal test was done by engaging the screw driver of the digital, torque meter (Lutron TQ-8800 Electronic Enterprise Co. Ltd. (ISO 9001 Quality management system certified by SGS) into the slit in the head of the implant to determine the peak torque necessary to unscrew the implant from its bed. The removal torque was expressed in Newton centimetre (N.cm).

Histological examination

For each healing interval (2 and 4 weeks) one animal was used for histological test. It was anaesthetized with anaesthetic 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 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 formalin for fixation, then left in a solution of sodium citrate and 10% formic acid to decalcify the bone. Finally, the specimens were molded in paraffin block then sectioning of 5-µm slice thickness, after placed on a slide. The slide placed in haematoxylin and Eosin stain to stain the tissue [25].

Statistical analysis

The suitable statistical analysis was done by using SPSS version 17, and these include:

1) **Descriptive statistics**
   a) Statistical tables.
   b) Summery statistic of the reading distribution (mean, SD, minimum, maximum).
2) **Inferential statistics**

a) Testing equality of means value by analysis of variance (ANOVA), this was used in order to accept or reject the statistical hypothesis and in comparisons, significant p-value was at ($p \leq 0.05$). LSD was used to compare the significance difference between means in this study.

RESULTS

***In vitro* part of study**

A. Phase identification

The XRD patterns of Cp-Ti specimen coated with a mixture of nano HA and $MgCl_2$ by electrophoresis deposition method and heat treated at 300°C in comparison with uncoated dental implant are shown in Figure 1. In this figure, the pattern (A) of uncoated Cp-Ti dental implant specimen shows strong line of Ti 002 and 101 at 2θ 38.42 and 40.17 respectively while the Ti mainly 110 at 2θ 38.48 was overlap by prominent peak of Ti 002 at 2θ 38.42.

The XRD pattern (B) shows strongest line of HA and $MgCl_2$ in the coated layer, and this is obvious from the 332, 161 and 300 planes for magnesium chloride $MgCl_2.12H_2O$ at 2θ, with following values respectively 36.6, 39.4 and 33.5 respectively and 300, 222 and 211 planes of HA at 2θ, 33.5, 47.5 and 32.3 respectively.

The peak indexing was carried out based on the JCPDS (Joint Committee on Powder Diffraction Standards) International centre for Diffraction Data, ICDD file # 44-1294 for titanium, # 9-432 for HA and magnesium chloride peaks reported #22-1147, Stoichiometric HA powders exhibited sharp diffraction peaks, indicating high crystallinity of the structure.

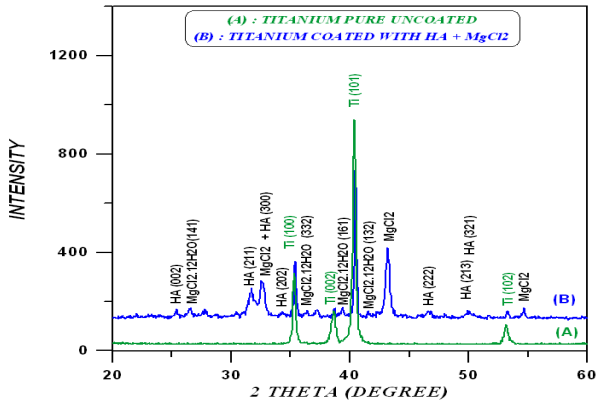
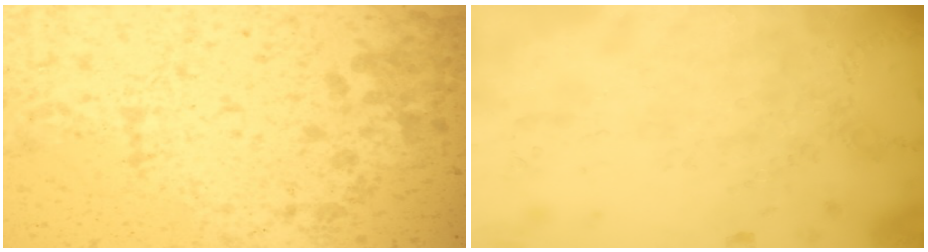


Figure 1 X-ray diffraction patterns of coated and uncoated specimen

B. Surface characteristics

1. Optical microscopical observations

The microstructure of HA/$MgCl_2$ coated Cp-Ti surface for different times (0.5, 1, 2, 3) min and at 30 V were imaged to determine the differences in surface morphology. In Figure 2, 2 min is suitable time used for coating screws at 30 V as it shows a homogenous, uniform thickness of coating without crack.



2 min (20x) **3 min (20x)**

Figure 2 Optical micrograph view of Cp-Ti plate coated with nano HA and $MgCl_2$ using 30 V in different times (20x)

Thickness measurement

The thickness of the coated layer was measured by the microprocess thickness gauge. The coating thickness of the coated film was increased with increasing of the time (Figure 3).

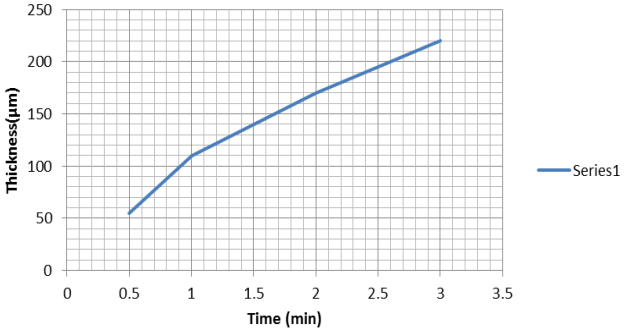


Figure 3 Thickness of nano HA and $MgCl_2$ film at 0.5, 1, 2, 3 min

SEM analysis

SEM images of Cp-Ti surface of coated specimen are shown in Figure 4. In this figure, coated plate showed numerous agglomerations of small spherical particles in nanometric scale. The coated plate surface showed difference in particles size or the degree of crystallinity of synthesized powder and continuous, uniform coating.

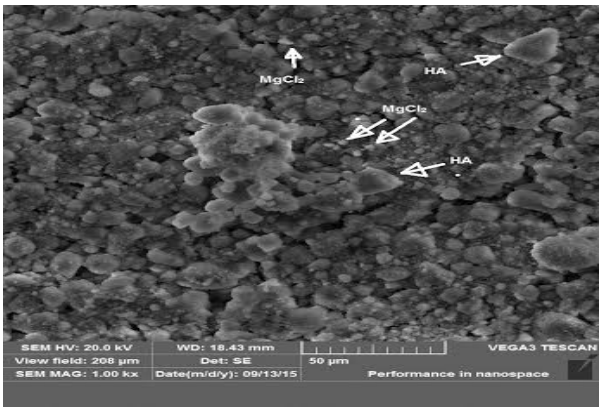


Figure 4 SEM Images surface morphology of coated specimens (HA/$MgCl_2$)

Elemental composition

Energy dispersive X-ray spectroscopy analysis showed that the main components of the coated plate were Ca^+ and Mg^+, as shown in Figure 5. The appropriate composition of materials was found to be homogenous all over the surface as screened by EDX analysis at different surface position and as shown in Figure 6.

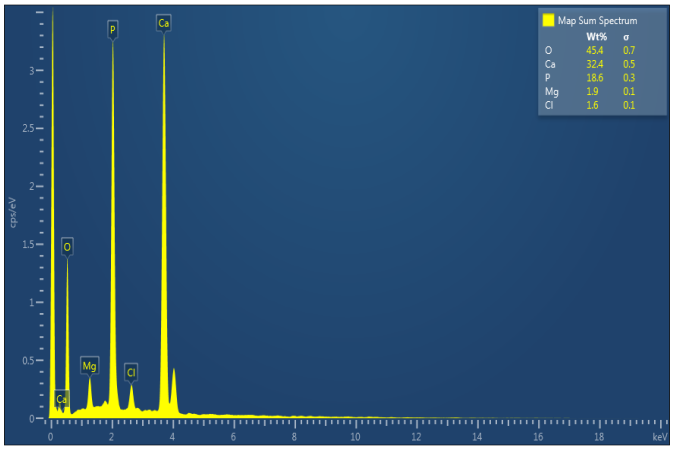


Figure 5 EDX image of elements coated Cp-Ti specimens (HA/$MgCl_2$)

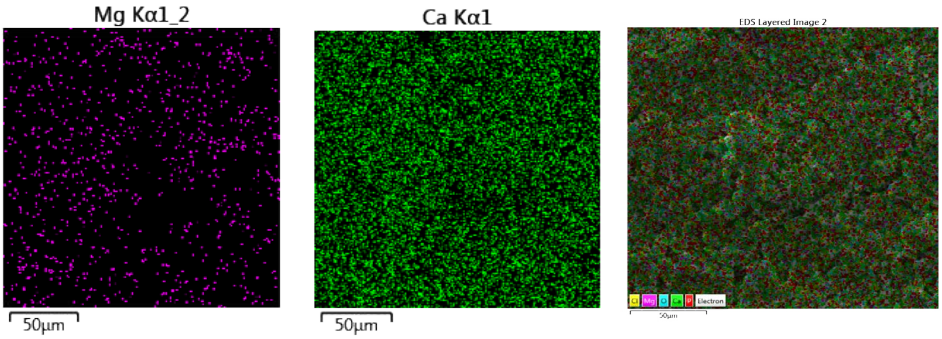


Figure 6 EDX map of the composition of coating materials HA/$MgCl_2$

Nano roughness surface analysis

In Figure 7 the nano roughness of nano Ti coated plate by electrophoresis method is shown, from this picture the specimen appears peaks and projections with the average roughness 86.89 nm and grain size 128 in nano particle size.

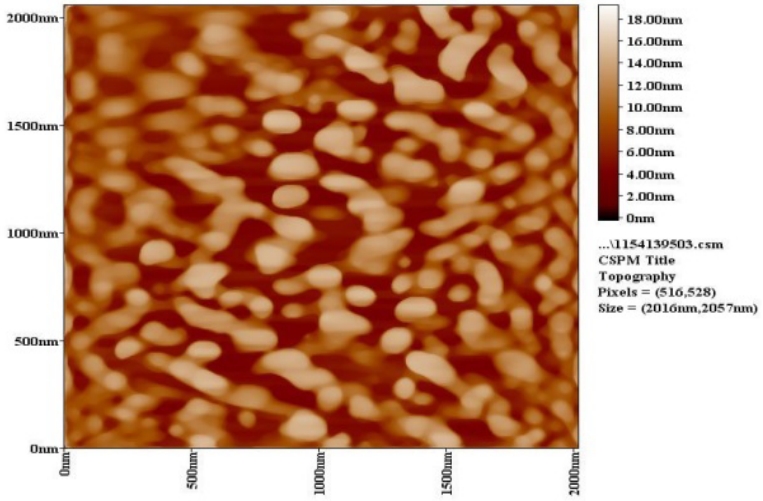


Figure 7 Topographic view of coated plate

***In vitro* study**

A. Mechanical testing

The removal torque mean value of different groups after 2 and 4 weeks of implantation time can be shown in Table 1. At time of 2 weeks, the torque val□ □ue that was needed to remove coated screws was higher than the uncoated screws with mean value (6.37 ± 4.480 N.cm). At time of 4 weeks the torque value that was needed to remove all the coated screws was higher than the uncoated Cp-Ti screws with mean value 11.40 N.cm. The quality of means between all groups of implants tested at 2 and 4 weeks of implantation were analysed by ANOVA test. Multiple Comparison among all groups of Cp-Ti screws along two periods of time independently shown in Table 2. In this table, there is a highly significant difference between uncoated and coated Cp-Ti screws at each period of healing intervals.

Table 1 Removal torque mean value of all tested groups after 2 and 4 weeks of implantation with ANOVA test

Groups	N	Mean N	Standard Deviation	ANOVA test	
				F -test	P value
Uncoated 2 weeks	8	4.48	0.77	50.099	0.000*
Coated 2 week	8	6.37	1.36		
Uncoated 4 weeks	8	8.4	0.68		
Coated 4 weeks	8	11.4	1.63		
*Highly significant					

Table 2 Least significance difference (LSD) test among different tested groups

Groups		Mean difference	P-value
Uncoated 2 weeks	Coated 2 weeks	-1.887*	0.003
	Uncoated 4 weeks	-3.912*	0.00
	Coated 4 weeks	-6.912*	0.00
Coated 2 weeks	Uncoated 4 weeks	-2.025*	0.002
	Coated 4 weeks	-5.025*	0.00
Uncoated 4 weeks	Coated 4 weeks	-3.000*	0.00
P ≤ 0.05 significant; P ≤ 0.000 Highly significant; P>0.05 nonsignificant			

B. Histological examination

Histological feature after 2 weeks of implantation

A. Uncoated dental implants: Histological feature of control group in Figure 8 shown a thread with apposition of new osteoid tissue and in a view (A), while (B) view illustrate osteoid tissue (woven bone) with blood vessel and osteoprogenitor cells scattered in bone marrow region.

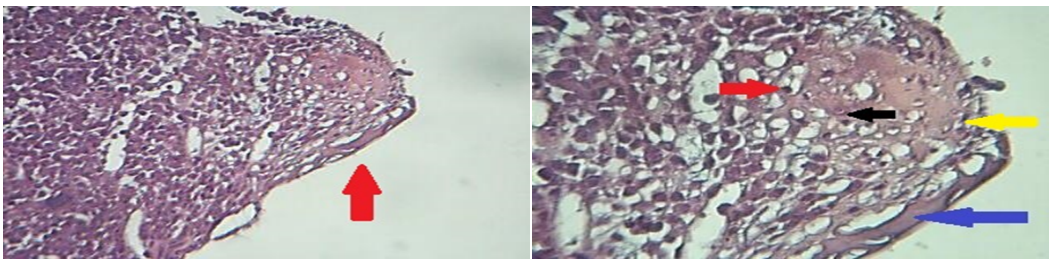


Figure 8 Photomicrographic view of uncoated screws after 2 weeks of implantation (A) Photomicrograph of threads, (arrow) Shows thread in bone marrow area H & E X20 (B) Magnifying view shows woven bone (Blue arrow) blood vessel (Black arrow), osteo-Progenitor cells (Red arrow), inflammatory cells, osteocytes (OS) (yellow arrow) H & EX40

B. Coated dental implants: The histological feature of coated group in Figures 9A and 9B shows threads developed within bone marrow with woven bone occupies apex of the thread, in A. In B view shows formation of new bone trabeculae with osteocyte and osteoblast around it.

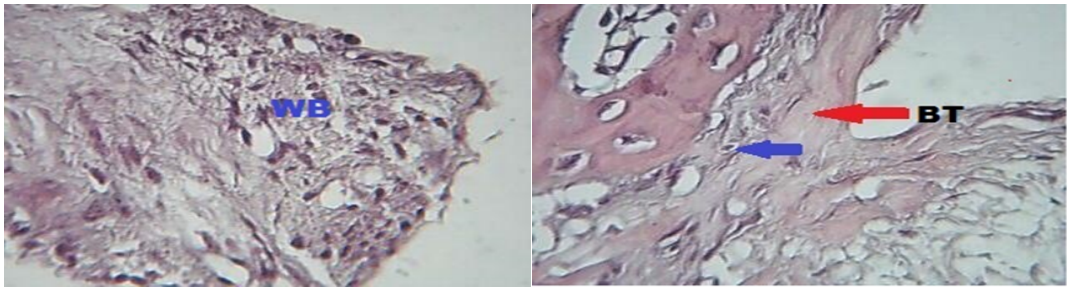


Figure 9 Photomicrograph view for coated group after 2 weeks of Implantation (A) Woven bone filled thread area H & EX20 (B) Numerous osteocytes (OS) (blue arrow) filled new bone trabeculae (red arrow) and osteoblasts (OB) around it H & EX40

Histological feature after 4 weeks of implantation

A. Uncoated dental implants

In Figure 10, photomicrograph view for control screws group at 4 weeks duration shows dens bone filled thread area surrounding by osteoblast and osteocyte detected in A and B.

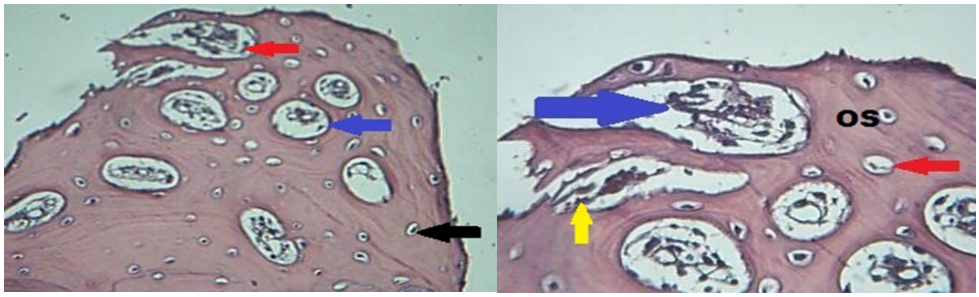


Figure 10 Photomicrograph view for control group after 4 weeks implantation (A) Shows dens bone filled thread area, osteoblast (red arrow), Haversian canal (blow arrow), osteocyte (black arrow) H & E X20 (B) numerous osteocytes filled bone trabeculae (OS) (red arrow) and osteoblasts (OB) (yellow arrow) and osteoclasts (OC) H & E X40

B. Coated dental implants

Figure 11 shows photomicrograph view of coated screws at 4 weeks duration in A there is a well mature bone filled thread area and reversal line and in B, the thread illustrates osteocytes (OS) arranged in circular way around Haversian canal.

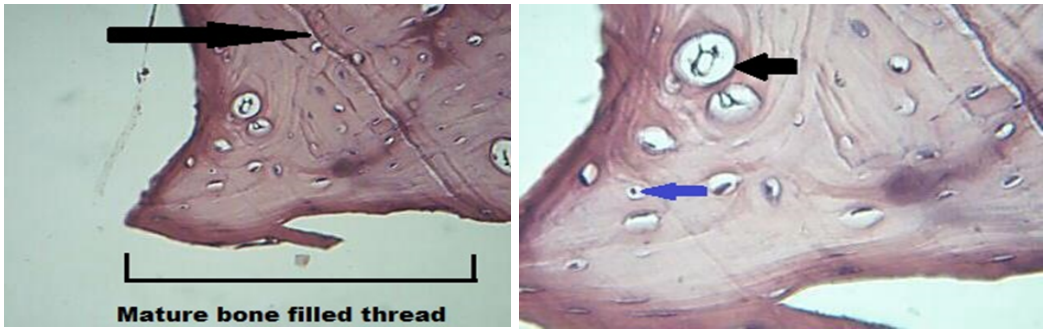


Figure 11 Photomicrograph view for coated screws group after 4 weeks of implantation (A) well mature bone filled thread area and reversal line (black arrow) H & E X10 (B) Osteocytes (OS) (blue arrow) arranged in circular way around Haversian canal (black arrow) H & E X40

DISCUSSION

In this study among various strains, adult New Zealand white male rabbits were selected to be used as an animal model. The tibia site in the rabbit were chosen to mimic the clinical situation. And since the dimensions of this bone correspond well with human alveolar space. Surgically this model provides low morbidity with easy access to the medical proximal tibia for implant placement. The morphologic characteristic of the rabbit tibia allows for implant fixture to engage cortical bone at its coronal aspect and marrow in the apical area. Also, tibia can be used as a suitable location for implant due to the presence of cancellous bone in addition to cortical bone. An animal of 2 kg to 2.5 kg weight since it had better capacity to withstand surgical trauma and less postoperative problems and leading to a better survival rate [26].

The age of the animals was from 10-12 months thus assuring complete closure of the proximal tibia epiphysis. And a more adult like bone physiology [27].

In the present study, EPD has been used for deposition of the mixture of (HA and $MgCl_2$), since that this method have several advantages of low cost, required simple equipment and the deposition of homogenous coating layer on complicated surface specimens (screw). Especially with the usage composite coating material [22,28].

EFD technique has been used in this study not only because of its cost effectiveness, requiring simple apparatus, but also it offers important role in the deposition of uniform thickness on substrates of complex geometry. This comes in agreement with Khor and Cheang in 2003 [29].

Surface feature (Optical microscopical findings)

The optical microscopical examination shows that the surface morphology for coated samples exhibit a fairly uniform distribution of particles, continuous, crack free and homogenous coating which indicate that the 2-min time coating at 30 V are suitable for composite material (HA and $MgCl_2$).

XRD phase analysis

HA/$MgCl_2$ composite have been successfully synthesized using the EPD method. XRD analysis and XRD indicated the phase purity and crystallinity of powder sample. It is evident from the figure of the XRD Figure 3 that the surface of the specimen is well covered with $MgCl_2$ because most of the diffraction peaks could be indexed to $MgCl_2$ corresponding to JCPDS (Joint Committee on Powder Diffraction Standards) file #22-1147. Also in addition specimen coated with HA is well covered because most of the diffraction peaks could be indexed to HA corresponding to JCPDS file #9-432.

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

As XRD shows that the narrower peaks are indicative of layer consists of highly crystalline form, whereas broad peaks represent lower levels of crystallinity, this comes with Jani [30].

Surface morphology and roughness (SEM and AFM)

In this study the average roughness (86 nm - 89 nm) and the average grain size 128 nm. This surface roughness estimated from the peaks that appeared on the surface and also the dimeter of the grains. This can explain that electrophoretic deposition technique is an efficient method in changing the surface feature of dental implant into nanoscale feature, this was agreed with Turky and Reffat [22,28].

***In vivo* experiments**

Animal description

Adult new white male rabbits were selected to be used as an animal model include the ease of manipulation and rapid bone healing response compared to other models. Also, the tibia site 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 characteristic of the rabbit tibia allows for implant fixture to engage cortical bone at its coronal aspect and marrow in the apical area [31].

The age of the animals was from 10-12 months thus assuring complete closure of the proximal tibia epiphysis. The requirement of a minimum age of 6 months ensures cessation of growth of the proximal tibia and a more adult like bone physiology, better approximating adult human bone [27].

Radiographic examination

In this study the radiographic examination, demonstrated a seemingly direct contact between bone and implant, there was no radiolucent zones or any abnormal reaction to the implant [32].

Clinical test

All animals show no sign of cross infection and tissue reaction or any type of negative clinical indicators as mobility of the screw were noted around the implant site. The absence of gross infection around the implant sight might indicate that the material was tolerated.

A perfect environment for implantation include sterilization of implant by gamma irradiation which is considered to be a superior method over the other like autoclaving, exposure to UV light or steam autoclave. Gamma irradiation used to sterilize all dental implant, this procedure commonly used in sterilizing biomedical materials and devices, which does not lead to significant change of the surface composition [33].

Mechanical test

In this study, the bone implant bond strength of Cp-Ti implants coated with HA nanoparticles and $MgCl_2$ was evaluated and compared with uncoated titanium implant. The removal torque was measured after 2 weeks of implantation in the rabbit tibia. statistical calculation showed a higher removal torque measurement value was needed to remove coated screws than uncoated screws (mean value for coated 6.37 N.cm, for the uncoated 4.48 N.cm) while after 4 weeks implantation (mean value for coated 11.40, for the uncoated implant screws 8.40) respectively with a highly significance difference.

The bone response, which means rate, quantity, and quality, are related to implant surface properties. For example, the composition and charges are critical for protein adsorption and cell attachment [34].

At the nano scale, a more textured surface topography increases the surface energy which in turn increases the wettability of the surface to blood, adhesion of cells to the surface, and facilitates binding of fibrin, matrix proteins, growth and differentiation factors. Nano topography, by modulating cell behaviour, can influence the process of cell migration, proliferation, and differentiation. These surfaces feature enhance the process of osseointegration by hastening the wound healing following implant placement [35].

After 4 weeks of implantation, a higher torque mean value was needed to remove coated screws than uncoated screws (11.40 N.cm and 8.40 N.cm) respectively. This indicates an increase in bond strength at the bone-implant interface in the coated with a significant difference in torque mean values between all groups of implants in different time periods.

Also, this significant increase of torque removal value due to continuous high activation of Mg^{+} Ca^{+} to osteoblast. than uncoated implants. The higher amount of new bone formed after 4 weeks which was transformed to mature bone together with the higher amount of new bone formed may reflect the higher bond strength at the implant bone interface and higher resistance to removal torque than uncoated implants.

The result of this study mentioned the torque mean value was increase as time progress this might be due to. The combination action of HA and $MgCl_2$.

First point, Hydroxyapatite begins to dissolve partially makes the surrounding fluids rich in calcium and phosphate ions, which seems to induce the precipitation of bone-like apatite on implant surface then bone-like apatite can trigger cellular differentiation and consequent bone formation (apatite plus osteoblast cells) [36].

Second, Due to the high activity of $MgCl_2$ is related to the high solubility. Magnesium has a very unique characteristic of dissolving readily in an aqueous solution that contains chloride ions [37]. One of the elements associated with biological apatite is magnesium. Mg incorporation into HA stimulates osteoblast proliferation. Mg acts similar to a growth factor during the early stages of osteogenesis and promotes bone formation [38].

Histological test

The histological analysis of all groups showed new bone trabeculae formation, with active osteoblast on borders. Also, it is clear from the obtained results that no inflammatory reaction was observed during the period of the implantation. This agrees with the results of Turky and Reffat [22,38].

This suggests that the woven bone formation began in the second weeks after placement. An osteoid tissue with numerous bones with progenitor cells around. The bone marrow showed active blood vessels, which indicate the beginning of new bone formation. These findings are supported by the work of Lins, et al., and Cooper [39,40].

After 4 weeks of implantation, the microscopical observation of uncoated implants showed more bone trabeculae formation filled the apex of thread and active proliferation osteogenic cells with active apposition of osteoid tissue. While the coated implants showed a well-developed bone close opposing to thread of implant, and area of immature new bone with harversian canals, filled base of implant impression bed.

After 4 weeks of implantation, by using light microscope we observe that the uncoated implant showed more bone trabeculae formation filled the apex of thread and active proliferating osteogenic cells with active apposition of osteoid tissue. While the coated implants showed a well-developed bone close opposing to threads of implant, and area of immature new bone also new osteoblast and osteocyte. It is suggested that rapid bone formation response to coating. Also new bone shows osteoblast and osteocyte. It is suggested that rapid bone formation response to the coating are dependent on better biocompatibility of the material which greatly affects the histological and biomechanical properties of the bone implant interface with no sign of inflammation.

Bone response (quality and quantity) are related to implant surface morphology and properties of materials coated layer. The use of HA which has the ability to stimulate bone formation by mixing with $MgCl_2$, Magnesium is essential for bone metabolism and stimulate new bone formation, and may also interact with integrins of osteoblasts which are responsible for cell adhesion and stability [41].

CONCLUSION

By using the electrophoretic deposition method can obtain a successful synthetic biocomposite coating nano HA/ $MgCl_2$ materials with homogenous and uniform thickness of coating. There is a highly significant increase in torque mean values after 4 weeks implantation as compared with 2 weeks and also significant increase in mean value in coated implant than uncoated one. Histologically, the use of a biocomposite material coated dental implant increase bone formation with time this mean better biocompatibility of nano HA and $MgCl_2$ coated on osseointegration of implant with bone bed.

DECLARATIONS

Conflict of Interest

The authors and planners have disclosed no potential conflicts of interest, financial or otherwise.

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