The Electrophoretic Deposition of Nano Al_2O_3 and $AgNO_3$ on CpTi Dental Implant (An *in vitro* and *in vivo* study)

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ABSTRACT

Background: Even the wide use of dental implants, still there is a proportion of implants are failed due to infection. Much considerable attention has been paid to modify the implant surface. Coating of dental implant with a biocomposite material of suitable properties can improve osseointegration. And this is the main concern of this study. The aim of present study was to evaluate the use of a biocomposite coating of dental implant with (ceramic nano Al_2O_3 and metalic $AgNo_3$) on the bond strength at bone – implant interface and tissue reaction.
Materials and methods: A total number of forty-eight screws, CpTi dental implant used in this study. Half of these screws were coated with a biocomposite material of nano ($Al_2O_3$and $AgNo_3$), this was done by using electrophoretic deposition method (EFD). In invitro part of the study, analysis of the coated surface was done using: X ray diffraction (XRD), atomic force microscope (AFM), Energy Dispersive X-ray spectroscopy (EDX), Optical microscopy and Scanning electron microscope (SEM). In invivo part of study, 10 white male New Zealand rabbits were used, and a screw type of dental implant [uncoated and coated with nano ($Al_2O_3$and $AgNo_3$)] were implanted in each tibia of rabbit. Then biomechanical and Histological test were performed after 2 and 4 weeks healing intervals.
Results: The results of biomechanical test showed a higher torque mean values of (M+SD):(14.91N.cm+2.7)and (22.5 N.cm +5.31) after 2 and 4 weeks respectively. In histological examination of coated screws at 2 weeks, there isa bone trabeculae occupies a base of implant bed with osteoblast andosteocyte. At 4 weeks, there is a progress in the healing process around dental implant, and this includes: -new bone with haversian canals, osteoblast and osteocyte.
Conclusion: Biocomposite coating of dental implant with Alumina and silver nitrate can be made by electrophoretic deposition method (EPD), and a multifunctional surface has been created.
Keywords: Electrophoretic Deposition, alumina, screw Dental Implants, torque. (J Bagh Coll Dentistry 2016; 28(1):41-47).

INTRODUCTION

Dental implants have many functions like support the crown, abutment of bridge and removable denture. A strong bond forms between bone and implant is an important factor in success of dental implant [1].

Various researches had been done for evaluationof tissue response to the implant surface and how can the characteristics of the surface, such as chemistry of surface, type ofcoatings and sterilization procedures can affect the long- and short- term stability of the metallo-biological interface [2-4].

However, it is difficult to meet all the requirements such as antibacterial ability, biocompatibility, osseointegration, and mechanical properties, but the essential factors for prolong stability of the implant are good biocompatibility and rapid osseointegration [5].

An aluminum oxide coating substrates showed improvement in corrosion resistance compared to uncoated titanium substrates [6].Ceramic materials such as alumina, partially stabilized zirconia, and titania possess high wear resistance, mechanical strength, and good biocompatibility [7]. When preparing implant sockets, the infection or trauma to the alveolar bone appears to be one of the causative factor of early implant losses [8], so researchers are increasingly focusing on the development of the antibacterial property of implants [9]. In literature, the usage of inorganic antibacterial materials give better results than those using of organic antibacterial materials, in the field of durability, toxicity and selectivity of action. Therefore, the benefit of (Ag ion) as an antibacterial agent has been known and Ag is currently used as antibacterial coatingsin several applications [10,11].

EPD 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 [12,13].

Electrophoretic deposition process is made directly by the application of an electrical field on a stable colloidal suspension. The wide using of electrophoretic deposition (EPD) method attracted much of interests that can be deposited with any size of a particle in powder form such as oxides, metals, polymers, carbides and nitrides [14-16]. In this study, using nano Al_2O_3 and $AgNO_3$ biocomposite coating CpTi for improvement of functionality and a biological efficacy of titanium implants.

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MATERIALS AND METHODS

In vitro study

Two types of a pilot was done, the first one is selection of the suitable suspension which was prepared according to the type of binder, either iodine or poly vinyl alcohol. And according to the result of pilot study, using suspensionof iodine found to be the best in terms of suspension stability and deposition features. This suspension consists of 4g nano alumina powder, 0.5g silver nitrate powder and 0.4g iodine, than these powders added to the solvent which was the 50ml 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.

A second pilot study was done to select suitable time used for coating. this pilot study consist of usingthree times which are (0.5 min, 1 min, 2min,and 3min) at 70V, the results showed thatthe use of 1 min for coating plats better in the term of homogeneity and uniform thickness of coating.

Analysis of coated surface was done by:-

X-ray Diffraction

Phase analysis was studied X-ray diffractometer using Cu Kα radiation. The 2 angles were swept from 20- 60° in step of one degree. The peak indexing was carried out based on the joint committee on powder diffraction standards (JCPDS).

Structural Surface Characterization

Scanning electron microscope was used for testing the nano surface feature as follows:

a- surface analysis

For studying the surface morphology and topographical characteristics of coated specimen.

1-Optical microscope was used forexamination of the surface feature of coated layer.

2- Scanning electron microscopy (SEM): This was used for examination of the surface in nano scale. It includes an electron beam scanned over the sample surface. The electron beam induces a larger depth of focus than a regular light beam and images at very high resolution can be recorded.

b- Material characterization

Energy-dispersive X-ray spectroscopy (EDX) analysis is 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 [17].

2-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 4weeks).

b. Experimental group (**16** screws): This group includes 8 screws for each healing interval (2 and 4 weeks) coated with ($Al_2O_3$and $AgNo_3$).

2. Histological test group: **(8** screws) in this test the screws were divided into:

a. Control group (**4** screws): include 2 screws for each healing interval (2 and 4weeks)

b. Experimental group (**4** screws):This group includes 2 screws for each healing interval (2 and 4 weeks)coated with ($Al_2O_3$and $AgNo_3$).

3- In vivo study

Ten healthy adult male rabbits weighing 2 -2.5 kg were used. The age of the rabbit was from 10-12 months. Animals were fed with standard pellets, jet and carrot and had free access to tap water. The rabbits were then left for two weeks in the same environment before the surgical operation.

A subcutaneous anti parasite agent (ivermectin) was given in a dose of 1ml injection. This was given to ensure parasite free animals.Also an antibiotic cover with oxytetracycline 20% (0.7ml/kg) intramuscular injection was given for 3 days to exclude any infection.A surgical procedure was performed according to **Helsinki** [18]. The total animals were dividedaccording to healing interval into 2 groups (2 and 4 weeks). At each time interval, one animal was sacrificed for histological study, and 4 animals were sacrificed for a mechanical test. All implants were implanted in tibiae of rabbit, each tibia received two implants (coated and uncoated) each rabbit was anesthetized by ketamine hydrochloride (1ml/kg B.W) and Xylazine 20mg/ml (1ml/kg B.W.)[19]. Both tibiae were shaved and cleaned with a mixture of ethanol and iodine.Later on, the incision through skin and fascia and muscles was made on the lateral side of rabbit's leg to expose the medial side of the tibia.

Bone penetration was performed witha serial of drills (2,2.5,2.8) by intermittent pressure with continuous cooling with normal saline.Coated screw was removed from an air tight plastic sheet, placed in the first hole (proximal one) using a screw driver first then a torque meter, so 5mm length of screw introduced in bone completely, then uncoated screw holds to second hole (distal one), thensuturing of muscles was done with absorbable catgut suture **3/0** followed by skin suturing with silk suture **3/0**. Postoperative care was performed by giving long acting systemic antibiotic (oxytetracycline 20%, 0.7ml/kg B.W.) for 5 days after surgery.

Mechanical Test (Torque Test)

Four animals were used forthis test. It was performed while the animal was anesthetized in the same manner mentioned in the implantation procedure.

Incision was made at lateral side of tibia then fascia and muscles were reflected to expose the implants. Tibia was supported firmly. A torque removal test was performed by engaging the head of torque meter (Dentium F28D104, Korea) into the slit in the head of the implant. The removal torque was expressed in Newton centimeter (N.cm).

Histological Test

For each healing interval (2-4 weeks) one animal was used for histological test.It was anaesthetized with anesthetic solution. Cutting of the bone around the implant was performed using a disk in low rotating speed hand piece with normal saline cooling.

Cutting was made about 5 mm away from the head of the implant to prepare a bone- implant block for histological study. Bone-implant blocks were immediately stored in 10% freshly prepared buffered formalin [20,21] and left for 3 days for fixation

RESULTS

A-In vitro part of study

<u>Phase identification</u>

The results of X-ray diffraction patterns of coated plates shown in figure 1. Then 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, #43-0649 for $AgNo_3$, #43-1484 for Al_2O_3

After EPD, it is evident from the figure that the surface of a specimen is well covered with $Al_2O_3$and $AgNo_3$,because most of the diffraction peak could be indexed to Al_2O_3phase according to JCPDS,The strongest line of this phase are (012),(110), (113), (024) and (116) at2 with the following values respectively (25.576), (37.767),(43.340),(52.548),(57.498) respectively.

The diffraction peak could be indexed to $AgNo_3$phase according to JCPDS, the strongest line of this phase are (113),(112),(213) and (215) at 2 (31.878), (24.871), (39.080) and (53.791)

Besides, the pattern showed the presence of Ti peaks (100), (101) and (002) at 2 (35.3376), (40.170) and (38.421) respectively. This is due to the penetration of X- rays beyond the coated layer

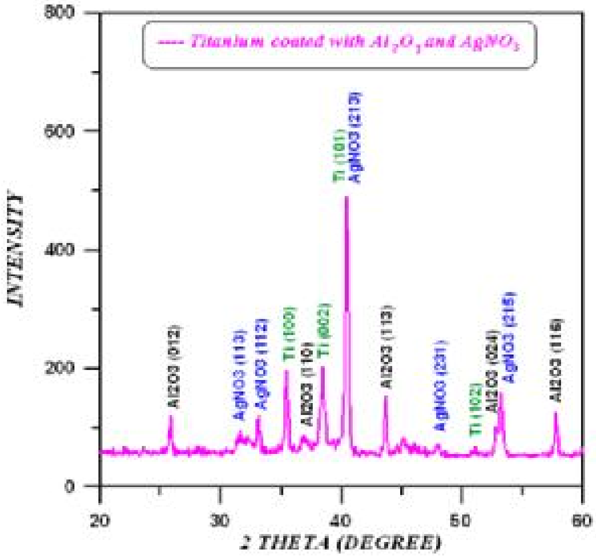

Figure1: X-Ray Diffraction Patterns of CpTi Specimen Coated with Al_2O_3 and $AgNo_3$.

<u>Nanosurface feature Morphological analysis (SEM)</u>

SEM micrographs of CpTi plate coated with Al_2O_3 and $AgNo_3$ showed that there were changes in the surface at low and high magnification. The SEM micrograph of coated sample shows many irregular projections; and the picture appeared that the surface had a feature or a structure of nano particle as shown in figure 2.

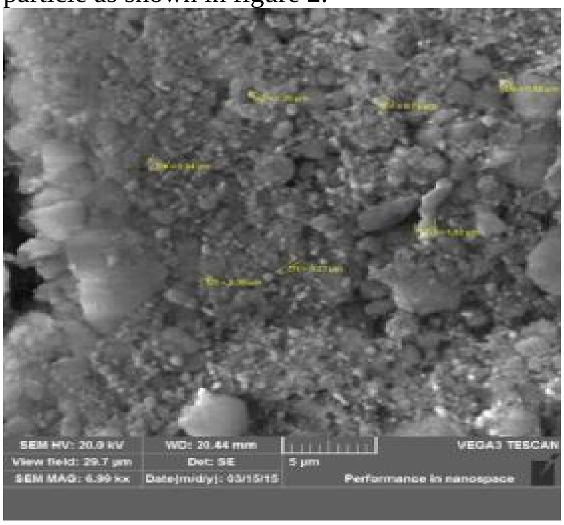

Figure2: SEM for Coated Plate Measures some of Particle Size

<u>Nanostructural characterization (Nano surface roughness analysis)</u>

Scanning probe microscope analysisshows peaks and projections with the average roughness **4.43 nm** as shown in **fig 3** and the average grain size **54.98 nm**.

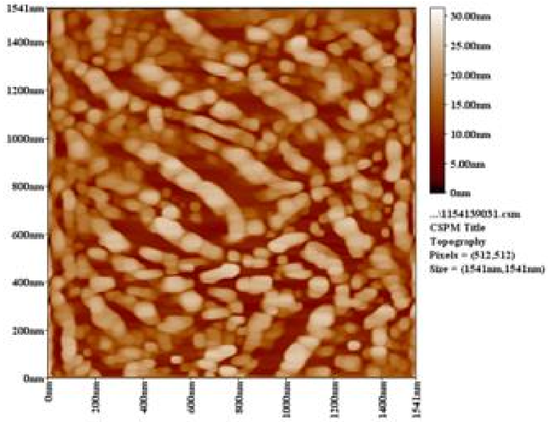

Figure 3: Average nano surface roughness of Al_2O_3 and $AgNO_3$coated CpTi dental implant.

<u>Elemental composition</u>

Energy-dispersive X-ray spectroscopy (EDX) analysis showed that the main components of the coated plate were Al and Ag as shown in **fig 4**. EDX spectra coated sample indicates the presence of small amounts of silver within the surface (7.1%) and 84.5% of Al^{+3}. The appropriate composition of coated materials were found to be homogeneous all over the surface as screened by EDX analysis at different surface positions as shown in **fig 5**.

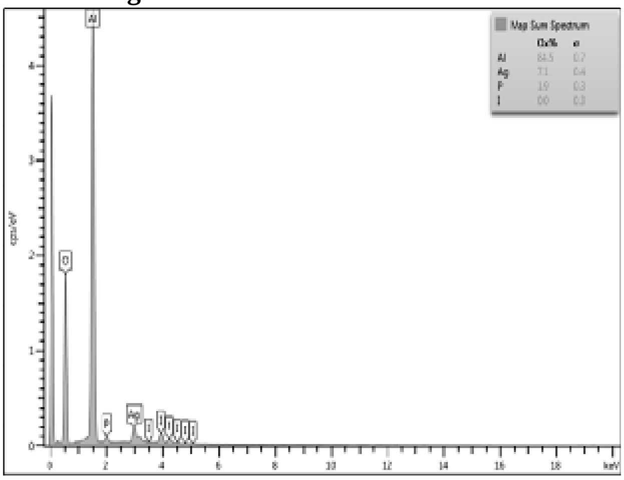

Figure 4: EDX-analysis of $Al_2O_3$and $AgNo_3$coatings on CpTi plate

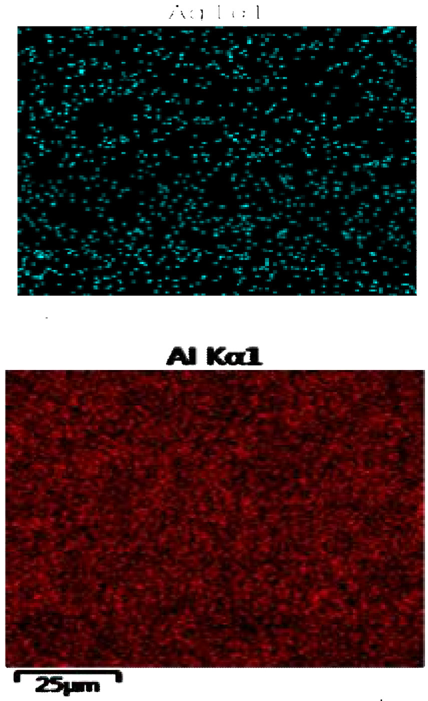

Figure5: SEM/EDX Mapping of Ag^+ and Al^{+3}

B-In vitro Part of Study

<u>Mechanical testing</u>

Descriptive statistics of removal torque values of CpTi screws coated with $Al_2O_3$and $AgNo_3$ after 2 weeks of implantation as shown in table 1, a higher torque mean value was needed to remove the implants coated with nano $Al_2O_3$and $AgNo_3$ (14.91 N.cm) as compared with the torque mean value of uncoated implant (10.75 N.cm) .

Also in table 1 descriptive removal torque mean values of CpTi screws after4 weeks of implantationrevealed, a higher torque mean value for the implants coated with nano $Al_2O_3$and $AgNo_3$ (22.5 N.cm) as compared to the torque mean value for uncoated implants (18.09 N.cm).

Table 1: Removal Torque Mean Values for Coated and Uncoated Implants at Different Time Intervals and ANOVA Test.

Time intervals	Groups	Mean N/cm	S.D	ANOVA	
				F test	P value
2 weeks	Coated	14.91	2.7	13.94	0.000
	Uncoated	10.7	2.2		
4 weeks	Coated	22.5	5.3		
	Uncoated	18.09	3.9		

Table 2: Multiple Comparison (LSD) among all Pairs of Different Periods of Healing Times in Each Group of CpTi Implant Screws Independently

Groups		Mean difference	Sig.
Control 2weeks	Coated 2weeks	4.16025	.035
	Control 4weeks	7.33563	.001
	Coated 4weeks	11.7488	.000
Coated 2weeks	Control 4weeks	3.17538	.103
	Coated 4weeks	7.58862	.000
Control 4weeks	coated 4weeks	4.41325	.026

Histological testing

After 2 weeks of implantation, in figure 6 the histological feature of nano$Al_2O_3$and $AgNo_3$ coated CpTi implants showed bone trabeculae (BT) occupies the apex of the thread and base of implant bed close to cutting bone (CB). In figure 7 a numerous blood vessels (arrow) with active proliferating osteogenic cells (arrow heads) occupies a base of implant bed.

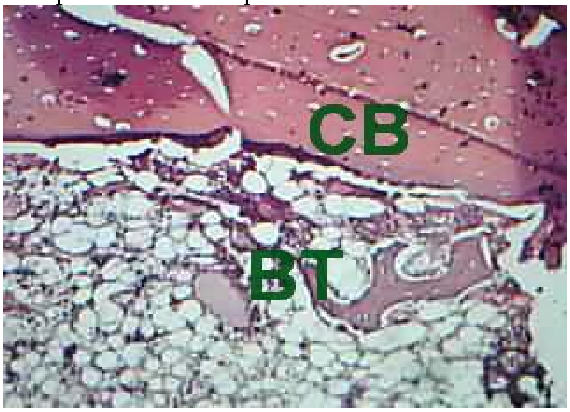

Fig 6: Microscopic Photograph View of$Al_2O_3$and $AgNo_3$ Coated Ti Implant after 2 Weeks of Implantation. Hand E×10

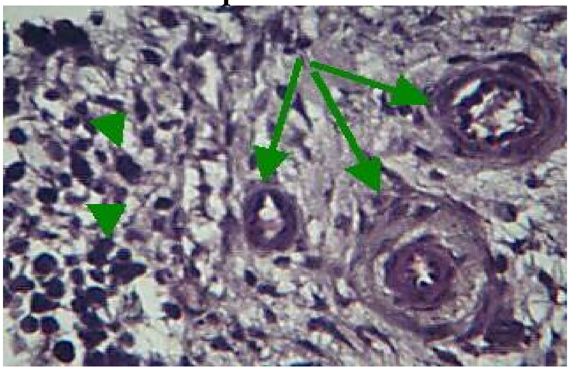

Fig 7: Microscopic Photograph View of $Al_2O_3$and $AgNo_3$ Coated Ti Implant after 2 Weeks of Implantation. Hand E×40

After 4 weeks of implantation, in figure 8 the histological feature of $Al_2O_3$and $AgNo_3$ coated CpTi implants illustrated immature new bone (NB) with haversian canals (arrow), filled base of implant impression bed. Also as shown in figure 9 new bone shows osteoblast (arrows) and osteocytes (arrow heads).

Fig 8: Microscopic Photograph View of Al_2O_3 and AgNO3 Coated Ti Implant after 4 Weeks of Implantation. Hand E×10

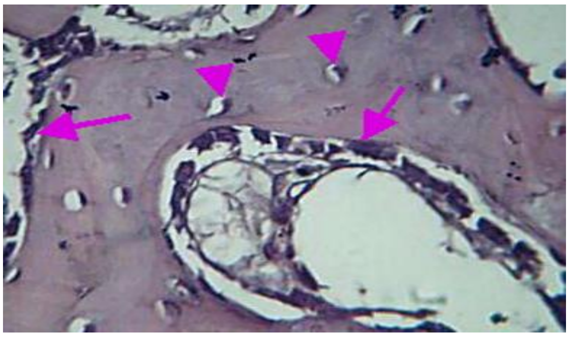

Fig 9: Microscopic Photograph View for the $Al_2O_3$and $AgNo_3$ Coated Ti Implant after4 Weeks of Implantation. Hand E×40

DISCUSSION

In this study, among various strains, Adult New Zealand white male rabbits were selected to be used as an animal model. This is for manipulation and rapid bone healing response as compared to other models, and these strains are less aggressive in nature and have less health problems as compared with other breeds [(22)].

The tibial sites in the rabbit were chosen to mimic the clinical situation, and since the dimensions of this bone correspond well with human alveolar space. Surgically, this model provides low morbidity with easy access to the medial proximal tibia for implant placement. The morphologic characteristics of the rabbit tibia allow for implant fixture to engage cortical bone at its coronal aspect and marrow in the apical area[(23)].

Tibia used as a suitable location for implant due to the presence of cancellous bone in addition to cortical bone. Also, it can provide a cushioning effect and prevents the cortical bone from splinting. It is better to choose a healthy large animal more than 2 to 2.5 kg since it had a better capacity to withstand surgical trauma and less postoperative problems and leading to a better survival rate [24].

The excellent mechanical properties and chemical stability of alumina encourage to be used as coated material on a metallic implant surfaces. Alumina coatedTi6Al4V can improve corrosion resistance of material and biocompatibility **[6, 25].**

In the field of research, it is well known that Nobel metals had biocompatibility and non-toxic to eukaryotic cells. One of these metals, silver hasa history in medical application as preventive effect of diseases and infections in little concentration **[26,27].**

In energy-dispersive X-ray spectroscopy (EDX) analysis, mapping of the coated plate showed a fairly uniform distribution of particles. In microstructural analysis, the main components of the tested plate were Al^{+3}85% and Ag^{+}7.1%. This can explain that the 5g of nano Al_2O_3 and 0.5g of $AgNo_3$ used in this study suitable for coated dental implant by the electrophoretic deposition method, especially when the mapping concentration can provide an antimicrobial effect on *Staphylococcus epidermis* and *Klebsiella pneumonia* [28].

In this study, a higher torque value was needed to remove coated screws than uncoated one, this was between 2 and 4 weeks of implantation, and the explanation of this might be due to theincrease in the bond strength at the bone–implant interface in coated implants. This agreed with the study of Salman [25]. Nanoscale topography of implant surface affectboth cell adhesion and cell motility and promote the osteoinductive molecular program for adherent osteoprogenitor cells, also nanoscale alterations may promote bone bonding behavior at bone implant interface [29,30].

The results mentioned that the torque mean value after 4 weeks of implantation higher significantly than 2 weeks of implantation, this indicated the progress of osseointegration leads to increase the bond between implant and bone. This come with the results of histological test which indicated a new bone formation with active proliferating osteogenic cell after 2 weeks of implantation, and immature new bone with havarsion canal filled the base of implant impression bed after 4 weeks of implantation, this indicate progress in healing of bone with time, that might lead to increase bond strength at bone-implant interface in the coated implant, also was suggested that the bone formation in response to the coating depend on better biocompatibility of the material which greatly affect the biomechanical properties at bone –implant interface with no sign of inflammation. This might be due to the presence of Ag which can promote bone formation. As silver has an antibacterial action on the coated surface, this make the process of bone formation earlier and rapid. This might be due to the presence of Ag which can promote bone formation.

The results of the present study strongly indicate that osseointegrtion can be obtained when coated implants are implanted in a living bone with a favorable biological environment for bone formation.

The biological significance of different healing reactions is of critical importance in attempting to unravel the role of surface material in ossointegration of bone-implant interface [31,32]. The histological analysis of all groups showed new bone trabeculae formation, with active osteoblats and osteocytes on borders. Also, it is clear from the obtained results that no inflammatory reaction was observed during the period of the implantation. This is agreed with the results of Yunzhi et al., [33].

From histological results of this study, the evidence of bone formation on coated CpTi implant suggests that the woven bone formation began in the second weeks after placement. Osteoid tissue with numerous bone 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 works of Lins et al., and Cooper[34,35].

From the results of the study, it can be concluded that:

1. It is can be successfully synthesise a biocomposite coating and a multifunctional surface of nano alumina and silver nitrate by electrophoretic deposition method (EPD), with homogenous and uniform thickness of coating.
2. Coating of dental implant with bio composite material results in a high torque removal mean values after 2and 4 weeks implantation, and there is a highly significant difference for the torque mean values after 4 weeks implantation as compared with 2 weeks with improved biocompatibility.

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