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Influence of Antimicrobial Bio-Composite Coating on Osseointegration of Dental Implant

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ABSTRACT

Background: *Prevention of surgical site infections at an early stage of implantation is an important goal for the long-term success of implants in dentistry. The aim of the study was to evaluate the mechanical properties of niobium-polyethylene glycol (Nb_2O_5-PEG 3500) biocomposite film formed by radio frequency (RF) magnetron plasma sputtering after immersion in antimicrobial glycol peptide (vancomycin).* ***Materials and methods:*** *RF magnetron technique was used to obtain a uniform and thin coating on commercially pure titanium substrates with certain sputtering parameters. Niobium oxide (Nb_2O_5) and niobium oxide\polyethylene glycol composite (Nb_2O_5\PEG) coatings were characterized by X-ray diffraction (XRD) for evaluation of structure and coating thickness measurement. The femur of 10 white New Zealand rabbits was selected as implantation site, each femur received two screws, the proximal screw was coated with Nb_2O_5\PEG composite film after immersion in the antimicrobial peptide (AMP) and the distal one was coated with Nb_2O_5. The mechanical assessment was performed to record new bone formation between the implant and original bone, after 2nd and 6th week healing periods.* ***Results:*** *Antimicrobial biocomposite coated implants showed a statistically significant increase in new bone formation in comparison to niobium oxide coated implants represented by an obvious increase in removal torque mean values at the 2nd week and 6th week after implantation.* ***Conclusion:*** *Coating commercially pure titanium implant with the antimicrobial biocomposite coating enhances the osseointegration at the bone-implant interface over the two periods of time.*

Keywords: Dental implants, Antimicrobial coat of implant, Antimicrobial peptide, Polyethylene glycol, RF magnetron sputtering

INTRODUCTION

Dental implants are a promising treatment modality for the replacement of missing teeth. Implant surface features are modified to obtain an adequate biological response in the bordering tissues [1,2].

Implant surfaces are classified into three major groups according to their biological response: bioinert, biotolerant and bioactive implant surfaces. The osteoconductive groups possess modified surface morphology that allows them to form new bone on the implant surface, these implant surfaces have different roughness and topographies that permit interaction with the proteins and allow migration of osteoblast precursor cells [3-5].

Various methods are applied to change the surface of the dental implants such as mechanizing, electropolishing, plasma spraying, coating, acid etching, surface oxidation, ionization, and phosphate deposit techniques. Surface modification of dental implants is considered as an optimal strategy in achieving rapid secondary stability, enhancing the bone-to-implant contact and reducing the time required for the replacement of missing teeth [6].

The surface roughness of the dental implant was created to enhance cell attachment. Ingrowth of tissue into the porous surface promotes osseointegration and mechanical interlocking of dental implants to the surrounding bone [7].

Biomaterials play an important role in the development of biomedical devices and implants. Several studies concluded that Nb possesses excellent biocompatibility and confirmed the possibility of using Nb films as coatings for implants [8,9].

Several studies found that surgical region infection is supposed to be acquired during surgery [10-12]. Multiple surface treatments have been applied to minimize the bacterial adhesion and biofilm formation.

Systemic or local prescription of antibacterial treatments focused on inhibiting bacterial infection has been applied. Systemic intake of antibacterial agents may have side effects while local application of antimicrobial agents may prevent the formation of biofilm around implants. Prophylactic coatings with the local application should possess sufficient release rate to prevent the bacterial adhesion or kill bacteria adhered to the implant surfaces and enhance implant success [11].

A new strategy for inhibition of implant-related infection includes coating implants with antimicrobial peptides, cytokines to stimulate host response to the bacterial infection [12].

Cationic antimicrobial peptides (AMPs) have been investigated in many studies. It has been revealed that AMPs act as a broad antimicrobial agent against both Gram-positive and Gram-negative bacteria, without inducing the development of resistant bacteria. Therefore, AMPs can be applied as a substitute for conventional antibiotics [13,14].

Antimicrobial peptides function by damage of the cell wall and inhibition of bacterial protein synthesis. In addition, they possess a great effect on inflammation, tissue healing because less bacterial resistance towards these prophylactic agents was noticed [15].

PATIENTS AND METHODS

In-vitro Experiment

Sample preparation: Grade 2 commercially pure titanium (Rod-shaped, 20 mm diameter) (Orotig Srl, company, Italy) was cut into small circular discs (20 mm diameter and 2 mm thickness) using Lathe machine. All specimens were grinded, polished and washed in alcohol, after that in deionized water to achieve a smooth mirror surface (Figure 1).

Figure 1 CPTi discs

Coating procedures: The radio frequency magnetron sputtering process was performed at certain parameters to obtain a thin uniform coating. Sintering of the Nb_2O_5 coated specimens at 700°C for 1 hour was carried out for densification using Carbolite furnace. The treatment is done under inert gas (argon), to prevent oxidation of the discs (Figure 2). While RF sputtering for deposition of biocomposite (Nb_2O_5\PEG 3500) was performed at different parameters (Table 1, Figure 3).

Figure 2 Nb_2O_5 coated CPTi disc

Table 1 Working conditions for deposition of Nb_2O_5 and Nb_2O_5\PEG biocomposite coating on CPTi substrate

Deposit	Power (W)	Pressure (Torr)	Substrate Temperature (°C)	Distance between target and substrate (cm)	Time of deposition (min.)
Nb_2O_5	75	$3x10^{-2}$	60	10	120
Nb_2O_5\PEG	50	$3x10^{-2}$	40	10	180

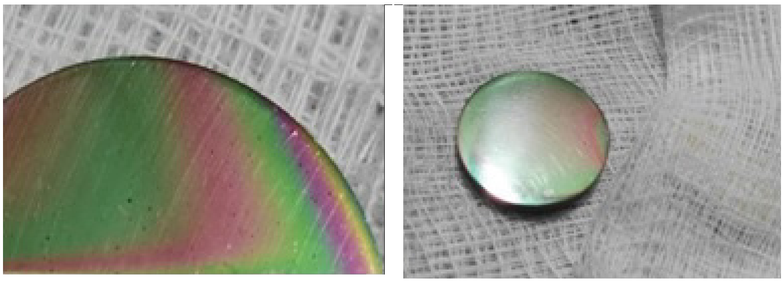

Figure 3 CPTi disc coated with Nb_2O_5\PEG biocomposite film phase analysis (X-Ray diffraction)

Phase and structural analysis were done on coated CP Ti discs by using X-ray diffractometer (Shimadzu LabX- XRD-6000). The 2θ angles were swept from 20°C-80°C in step of 2 degrees per minute. The peak indexing was carried out based on the JCPDS (joint committee on powder diffraction standards) files.

Coat thickness measurement: The cross-section of coated discs was utilized for measurement of the coating thickness of the deposited film by field emission scanning electron microscope analysis (FESEM). The thickness of both Nb_2O_5 and Nb_2O_5 \ PEG (3500) biocomposite coatings was measured.

***In-vivo* Experiment**

Implant preparation: Total 40 screws were prepared from CpTi rods using Lathe machine, the length of the screw was 8 mm and the diameter was 3 mm (threaded part was 5 mm and smooth part was 3 mm) with pitch height of 1 mm. A slit was prepared on the smooth side of the screw to fit the torque meter. Around 20 coated screws were prepared for each healing interval (Figure 4).

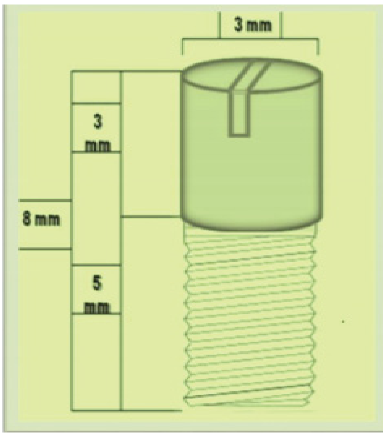

Figure 4 CPTi screws

Surgical procedure: Around 10 adults white New Zealand experimental rabbits were used, their weight range from 1.8 Kg to 2 Kg. The age of the animals was from 10-12 months. The animals were anesthetized with a combination of ketamine (1 ml/kg) and xylazine (1 ml/kg) intramuscularly. The required dose of anesthesia and antibiotic was calculated by weighting each rabbit. The femur was shaved by shaving spray, washed with iodine. The incision was made through skin, fascia, muscle, and periosteum to expose the bone. Round drill with 2 mm diameter was used to make two holes, 10 mm apart with intermitted pressure at a rotary speed of 1500 rpm and continuous normal saline cooling. The implant bed enlargement obtained gradually with spiral drills until size 3 mm. The biocomposite coated screw was immersed in AMP (6%) and placed in the first hole (proximal one). The niobium oxide coated screw was inserted in the second hole (distal one). The final tightening was done with torque meter (approximately 10 N.cm). Then catgut suture 3/0 was utilized for suturing the muscle followed by using silk suture 3/0 for skin suturing, local antibiotic performed over the wound site, followed by systemic antibiotic injection (oxytetracycline 20%) for 5 days to obtain an infection-free surgical site. After 2 and 6 weeks of healing intervals, the coated screws were removed using a digital torque meter (Lutron TQ-8800) to record removed torque values.

Statistical Analysis

Statistical analysis was done using one-way ANOVA and Tukey HSD tests. A $p<0.05$ was considered as a level of significance.

RESULTS

***In vitro* Experiments**

Phase analysis of Nb_2O_5 film: Figure 5 shows XRD patterns for CPTi specimen coated with Nb_2O_5 by RF plasma sputtering for 120 minutes before and after heat treatment. The pattern shows main peaks of the crystalline structure of Nb_2O_5 indicating that the CPTi substrate was well covered by an oxide film.

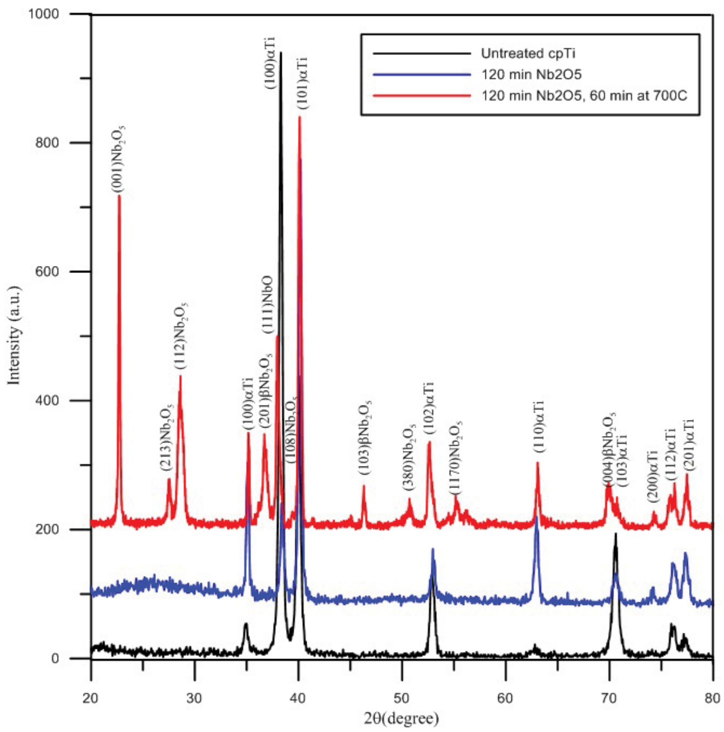

Figure 5 XRD spectroscopy of Nb_2O_5 coating at 120 min magnetron plasma sputtering

Phase analysis of Nb_2O_5 /PEG film: Figure 6 presenting the X-ray patterns of CPTi substrate coated by Nb_2O_5/PEG composite in comparison to the untreated one. The patterns show clear peaks (332), (523), (827) and (382) which belong to Nb_2O_5 phase at 2θ 35.400°C and 71.050°C, 53.350°C, 63.700°C respectively. Also, a new prominent peak clearly can be seen at 2θ 39.550°C and 57.200°C which belongs to the (512) and (715) Nb_2O_5 phase respectively.

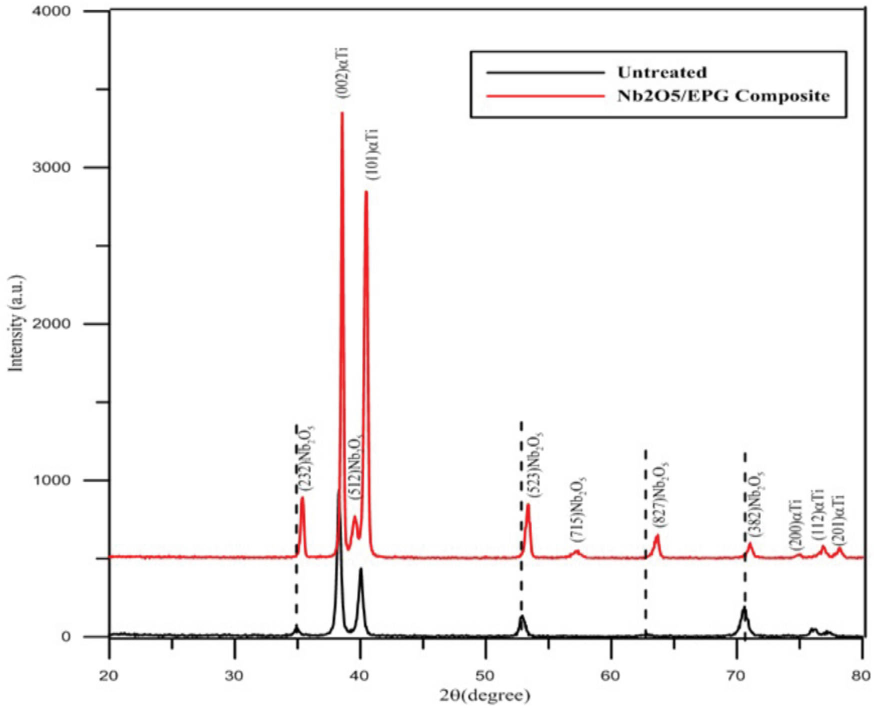

Figure 6 XRD patterns of CPTi substrate coated with Nb_2O_5/PEG composite

Nb_2O_5 and Nb_2O_5/PEG thickness Measurement: Figure 7 shows micrograph taken by Field emission scanning electron microscope which was used for the assessment of Nb_2O_5 thin film thickness. It was found that the Nb_2O_5 deposited layer is 121.63 nm in thickness. The thicker composite film was formed on a CPTi substrate with a thickness of 637.87 nm which can be seen in SEM micrograph (Figure 8).

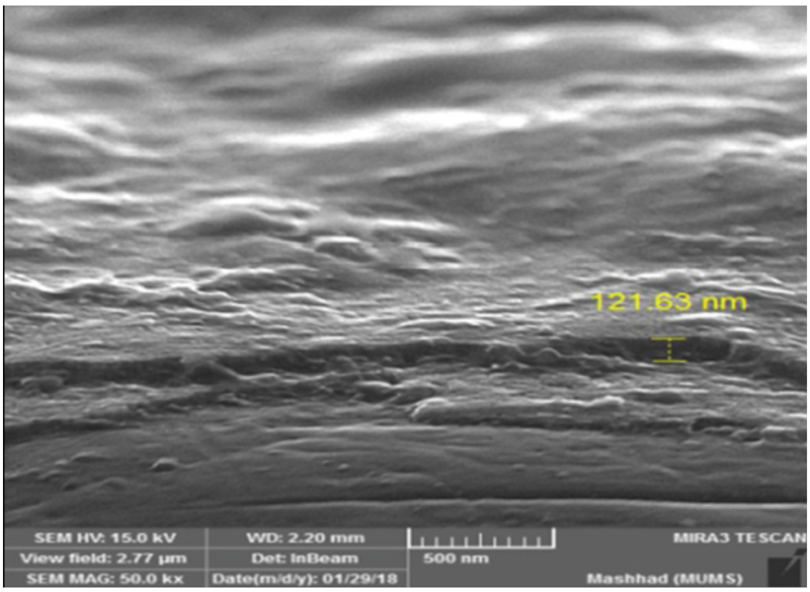

Figure 7 SEM micrograph demonstrated Nb_2O_5 coat thickness

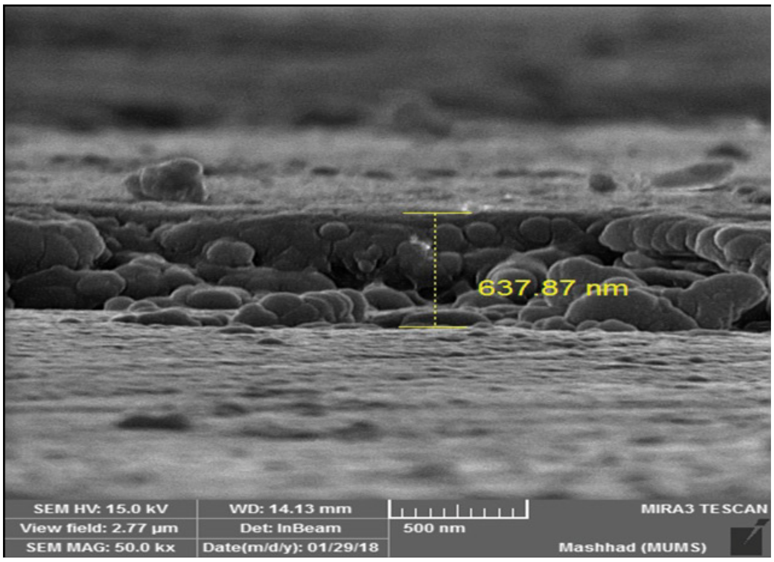

Figure 8 SEM micrograph demonstrated Nb_2O_5/PEG biocomposite film thickness

***In-vivo* experiments**

Mechanical evaluation of implanted screws: The descriptive and summary of the differences in the torque mean among all groups was revealed in Table 2. The results exhibited that after, removal of torque mean value of biocomposite (Nb_2O_5\PEG) coated implants following immersion in glycopeptide vancomycin was higher (17.650 N.cm) in comparison to Nb_2O_5 coated screws (16.430 N.cm), also higher removal torque mean value for (Nb_2O_5\PEG) biocomposite coated implants following immersion in glycopeptide vancomycin was found (22.740 N.cm) after 6 weeks healing period in comparison to Nb_2O_5 coated screws (20.341 N.cm). ANOVA analysis revealed that coating the implant surface with biocomposite film after immersion in glycopeptide significantly increases the removal of the torque mean values.

Table 2 Comparison of torque mean values of experimental groups

Groups	Mean	SD	SE	Minimum	Maximum	F	Sig.
Nb_2O_5 2 weeks	16.43	2.146	0.679	13.4	19.8	25.035	0.000 HS
Nb_2O_5 6 weeks	20.34	1.839	0.581	18.2	23.8		
Nb_2O_5\PEG biocomposite + AMP 2 weeks	17.65	1.177	0.372	15.7	19.2		
Nb_2O_5\PEG biocomposite + AMP 6 weeks	22.74	1.829	0.578	19.8	25.6		

Tukey analysis showed a statistically significant increase of removal torque value for antimicrobial biocomposite coated screws after 2nd week in comparison to 6th week implanted Nb_2O_5 coated screws, also the results exhibited a non-significant increase of removal of the torque value for antimicrobial biocomposite coated screws in comparison to Nb_2O_5 coated screws at 2nd week healing interval (Table 3).

Table 3 Tukey HSD analysis among the experimental groups

(I) Groups	(J) Groups	Mean Difference (I-J)	Sig.
Nb_2O_5 2 weeks	Nb_2O_5 6 weeks	-3.91	0.000
	Biocomposite + AMP 2weeks	-1.22	0.431
	Biocomposite + AMP 6 weeks	-6.31	0.000
Nb_2O_5 6 weeks	Biocomposite + AMP 2 weeks	2.69	0.009
	Biocomposite + AMP 6 weeks	-2.40	0.023
Nb_2O_5\PEG biocomposite + AMP 2 weeks	Biocomposite + AMP 6 weeks	-5.09	0.000

DISCUSSION

In-vitro Experiments

Enhanced bone bonding and increased bone formation tend to be possible with roughened surfaces modified using certain surface treatments. RF magnetron sputtering deposited a thin, crystalline and rough coating on titanium substrate [8]. Many authors concluded that microsurface roughness tends to enhance the osteoconduction (in-migration of new bone) around the implant surface by using implant as a source for local delivery of bioactive agents [16,17].

RF magnetron sputtering produced nanoscale thickness films which could improve the adhesive strength of the coating, this finding is in agreement with several studies that explained the relation between coating thickness and adhesion, they found that thicker coatings have to debond from their substrates [8,18,19].

In-vivo Experiments

Mechanical test: Removed torque was used in this study as a parameter for osseointegration. Torque is 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 [20-22].

Effect of healing intervals on removed torque test: The present results demonstrated that there was a clear enhancement in the removal of torque value with time which may be due to progressive bone growth in bone-metal contact and remodeling around the implant during healing period that subsequently improved the mechanical interlocking of (Nb_2O_5) and (Nb_2O_5\PEG 3500) biocomposite coated implant. This finding agreed with many authors [17,18,23]. The force required to remove the implant from the living bone was greater with the increased implantation time. This could be related to increased shear strength, which resulted in stress transfer from the implant to the bordered bone, an even stress distribution between the implant and living bone, reduced stresses in the implant and thereafter improved osseointegration [24].

Effect of coating materials on removal torque test: Antimicrobial niobium oxide-polyethylene glycol (Nb_2O_5\PEG 3500) biocomposite coated screws recorded statistically higher mean value of removal torque in comparison to Nb_2O_5 coated screws at 6 weeks healing period. This indicated an increased bond strength at the bone-implant contact, this finding was in agreement with Al-Mudarris who concluded that both the surface morphology and oxide thickness influence the bone response to titanium [22,25]. Also, this finding could be attributed to that of the new bone transformed to mature bone at 6th week, the higher amount of new bone formation may demonstrate the higher bond strength at the implant-bone interface and higher resistance to removal torque.

Two weeks implanted antimicrobial (Nb_2O_5\ PEG 3500) biocomposite coated screws showed highly significant increase in removal torque value in comparison to six weeks implanted Nb_2O_5 coated screws, this finding proved that the biocomposite coating could shorten the healing period for dental implants and benefit both patients and dentists, this finding agrees with the previous studies which found that a thin layer of antimicrobial peptides attached to the surfaces of dental metal alloys showed excellent antibacterial effects against typical pathogens related to surgical site infection, consequently healthy implant bed enhanced osseointegration [13-15].

The non-significant difference of removal of torque values of antimicrobial biocomposite oxide coated screws in comparison to niobium oxide coated screws at 2 weeks healing period could relate to the uncalcified newly formed bone.

CONCLUSION

In conclusion, surface modifications on implant surfaces enhanced osseointegration. However, the use of Nb_2O_5\PEG 3500 biocomposite after immersion in AMP showed the increased removal of torque at two healing intervals.

DECLARATIONS

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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