Mechanical evaluation of nano hydroxyapatite, chitosan and collagen composite coating compared with nano hydroxyapatite coating on commercially pure titanium dental implants

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ABSTRACT

Background: Dental implants act as infrastructure for fixed restoration to look like as a natural tooth. Osseointegration is a biological events and considered as a base for success of dental implant. The aim of this study is to evaluate the bond strength between bone and Ti implant coated with mixture of nano hydroxyapatite-chitosan-collagen compared with Ti implants coated with nano hydroxyapatite implanted in rabbit tibia, after different period of implantation time (two and six weeks) by torque removal test.

Material and methods: 36 screws of commercially pure titanium; 8mm in length and 3mm diameter , 18 screws coated with mixture of nano hydroxyapatite-chitosan-collagen and18 screws coated with nano hydroxyapatite by dip coating. Structural characteristics was assessed by scanning electron microscope, and FTIR analysis. The screws were implanted in 18 healthy adult male New Zeeland rabbits each tibia received one screw, right tibia received screw coated with nano hydroxyapatite while left tibia received screw coated nano hydroxyapatite-chitosan-collagen composite. Removal torque test was done by torque meter to determine the highest torque value necessary to remove the implants from tibia bone after different period of time of implantation(2 and 6 weeks).

Result: Nano hydroxyapatite-chitosan-collagen composite coating was resulting in higher torque removal value than nano hydroxyapatite coating for two periods of time.

Conclusion: Concluded that addition of collagen and chitosan to nano hydroxyapatite was more efficient in rapid bone formation than nano hydroxyapatite only.

Keywords: Osseointegration, nano hydroxyapatite, chitosan, collagen, FTIR analysis. . **(J Bagh Coll Dentistry 2017; 29(2):42-48)**

INTRODUCTION

Dental implant was considered an accepted alternative treatment to restore missing teeth and tissues. Titanium shows an excellent biocompatible nature and minimum foreign body reaction in comparison with other conventional materials [1, 2]. During the past decades, many researchers suggested that increasing the local quality and quantity of the surrounding tissue for favorable osseointegration [3].

Many studies have concentrated on finding methods to increase and improve osseointegration, providing adequate mechanical integrity to resist occlusal forces at an early period [4, 5].

Bone involve organic and inorganic material within extracellular matrix, the organic constitute is the collagen, the tensile strength of bone is contributed to collagen fiber. While the inorganic component of bone is the hydroxyapatite [6]. Amongst other types of calcium phosphate, hydroxyapatite considered the best bioactivity. Hydroxyapatite (HA) has ability to improve adhesion, migration, differentiation and proliferation of osteoblast; which is important for bone renewal [7].

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Chitosan is nontoxic biocompatible biomaterial with antimicrobial activity, so the chitosan is widely used in biomedical application [8]. The abundant protein in the animal kingdom is the collagen, collagen forming about 25% of the total protein content of the body [9]. The purpose of this study was to evaluate the effect of mixture from nano hydroxyapatite, chitosan and collagen composite coating compared with nano hydroxyapatite coating materials on the strength of bone-implant interface after implantation in rabbit tibia bone by means of torque test.

MATERIAL AND METHODS

Sample preparation

Commercially pure titanium discs (grade 2), 29mm diameter and 2mm thickness was used as substrate for coating, These Ti discs 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[10]. After that the specimens left to dry at room temperature to be used in the pilot study

Pilot study

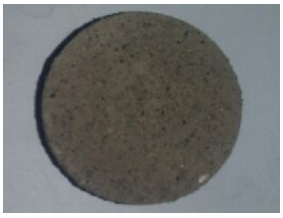
1-Coating solution preparation

Eighteen screws coated by nano HA by dip coating (nano HA solution prepared by dissolving0.01g of P_2O_5 in 50ml of ethanol, after half an hour of stirring on hot stirrer, add 7 g of

nano HA powder ,then sintered to 400ºC under inert gas (argon) [9]. Eighteen screws coated with nano HA-chitosan-collagen mixture by dip coating for 2 minutes (0.5 g of chitosan dissolved in 50 ml of 2% acetic acid then add 1µg/ml of collagen then this solution mixed with nano HA solution that prepared as 4g of nano HA dissolved in 50 ml of absolute alcohol (ethanol)).

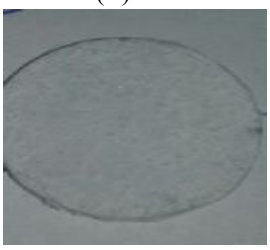
2- Heat treatment

Heat treatment(sintering) for densification of coated discs by using carbolated furnace, heat treatment done for one hour under inert gas, for nanohyroxyapatite coating substrate, best heat treatment was at 400ºC [10]. While the nanohyroxyapatite, chitosan and collagen composite coating, best heat treatment was at 100ºC. because heating above this temperature was resulted in burning of coating material as shown in figure(1).



(A)

(B)



(C)

(Figure1): heat treatment (A) heat treatment at 300ºC. (B) Heat treatment at 150ºC. (C) Heat treatment at 100ºC.

Test performed on coated Ti discs

1- FTIR analysis

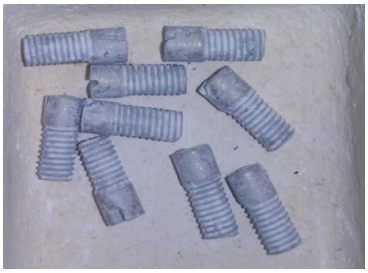
FTIR analysis was used to identify the organic and inorganic materials, within scanning range between 400 to 4000cm^{-1}.

2- Scanning Electron Microscope (SEM)

SEM analysis was used for evaluating the surface morphology and topographical characteristics and particle size of coated substrate.

Implant preparation

Commercially pure titanium (grad2) rod 6 mm diameter, shaped by lathe machine into screw shape implants, thirty six screws shaped implants,3 mm diameter and 8mm length (threaded part is 5mm, 3mm length of smooth part), with slit in the head of implant, 1mm depth to fit the screwdriver during implantation. These screws washed in ethanol in an ultrasonic cleaner for 15 minutes to remove the debris and contamination, then dried at room temperature, after that coated according to pilot study results as shown in figure (2).



(Figure2): coated screws.

Surgical procedure

Eighteen healthy adult male New Zeeland rabbits 10-12 months age and 2-2.5 kg weight were used. Before surgical operation, the rabbits were left in same environment with antibiotic cover by oxytetracycline intramuscular injection to exclude any infection before operation. Anesthesia was given to rabbits by intramuscular injection of Ketamine hydrochloride (1ml/1Kg body weight) and xylocaine 2 %(1ml/1Kg body weight), so each rabbit must weighted before surgical procedure to determine appropriate amount of anesthesia which must be given to each rabbit.

After shaving of skin and cleaning with alcohol, incision was made on the medial side of tibia then reflection must made to expose the bone, handpiece used to prepare hole on the bone (2.5mm in diameter), drilling must be gently with continuous cooling to prevent damage the bone.one hole prepared on right tibia to insert nanoHA coated screw within it, and one hole on the left tibia to place the nanoHA-chitosan-collagen mixture coated screw. Muscles sutured by absorbable catgut suture, while skin sutured

by silk suture. After suturing local antibiotic (oxytetracycline spray) applied to surgical site, and systemic antibiotic was also given. Rabbits remain under cover of antibiotic (local and systemic) for three days after operation. After specific period of time the rabbits anesthetized with Ketamine hydrochloride (1ml/1Kg body weight) and xylocaine 2 %(1ml/1Kg body weight) the stability was accomplished by placing the torque meter into the slit in the head of implants to determine amount of torque required for removing the screws to assess implant-bone contact.

Mechanical testing

Removal torque test used to determine amount of force required to remove implanted screws after different period of healing by using of torque meter (STURTEVANT RICHMONT TORQUE PRODUCT, MODEL F 80-1-0. USA. 0-80 inch. ounces, with accuracy ±2%). The removal torque value was expressed in Newton centimeter (N.cm).

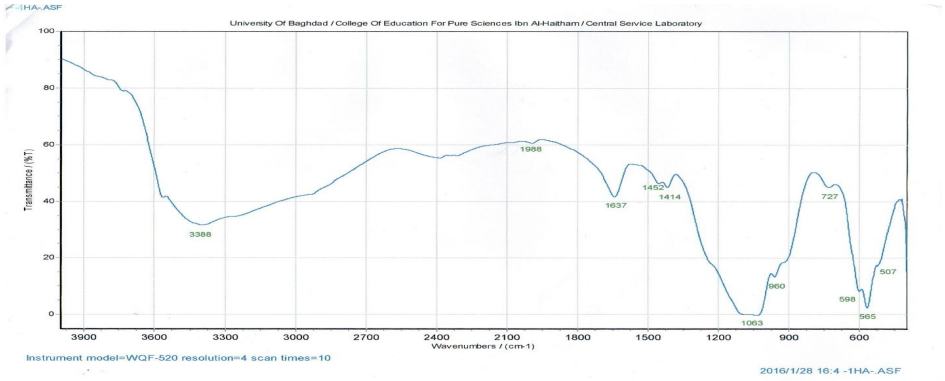
RESULTS

FTIR analysis

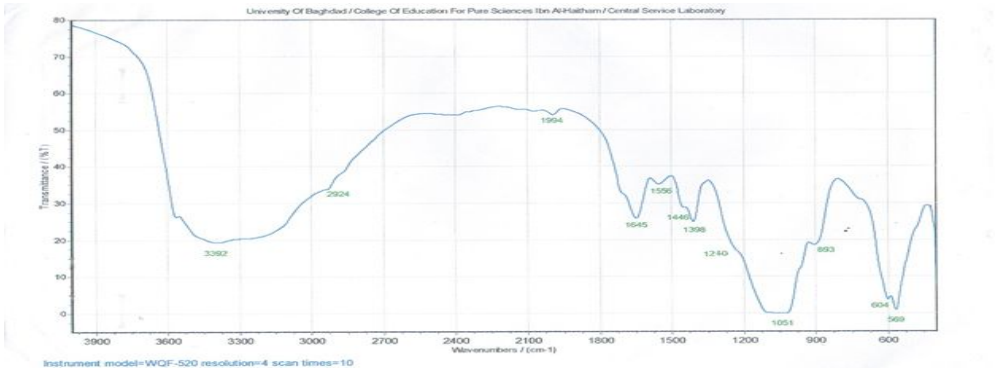
Result of FTIR of nanohyroxyapatite (figure3) was showed spectra at 565,598,980,1063cm^{-1}correspond to PO_4^{-3} of HA, peak at 1452cm^{-1}correspond to CO_3 of HA [11, 12]. Band at 1637 due to absorbed water.

While FTIR of nano HA-chitosan-collagen mixture (figure 4) was showed shifting of PO_4^{-3} group of HA into 569,604 and 1051.

Spectra at 1240, 1556 and 1645 correspond to amide III,II,I respectively of collagen [13]…while band at 2924 belong to asymmetric stretching of CH3 of chitosan [14].and band at 3388cm^{-1} was belong to NH group stretching vibration of chitosan [15].



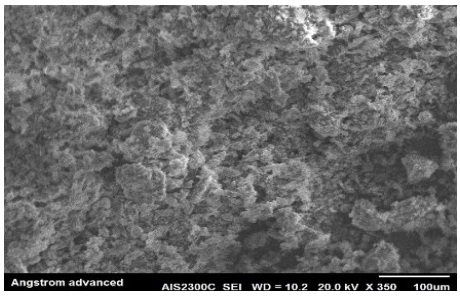
(figure3): FTIR of nano HA



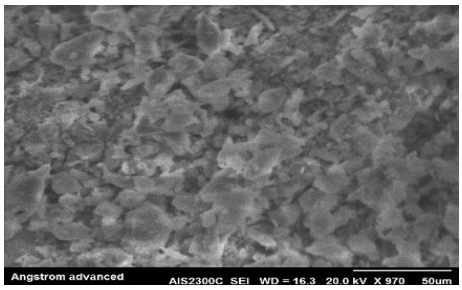
(Figure 4): FTIR of nano HA-chitosan-collagen mixture coating

Scanning Electron Microscope (SEM)

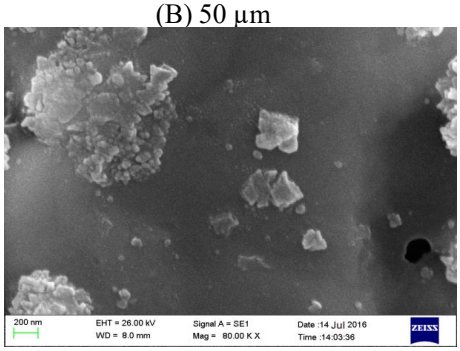
SEM images of nanohyroxyapatite coating (figure 5) and nanohyroxyapatite, chitosan and collagen composite coating (figure 6). It shows uniform coating with particle size of about 200nm in size.



(A) 100µm

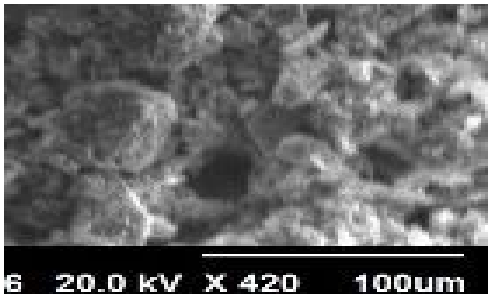


(B) 50 µm

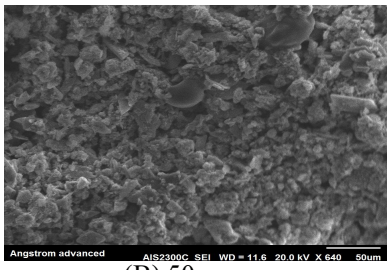


(C) 200nm

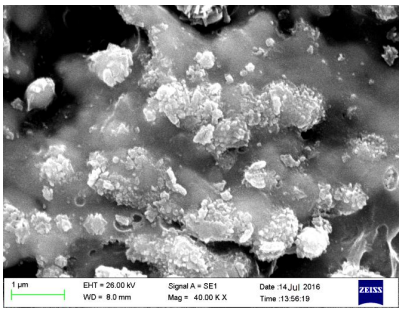
(Figure5 A, B, C): SEM of nano HA coating at different magnifications.



(A)100µm



(B) 50µm



(C) 200nm

(Figure 6 A, B, C): SEM of nano HA-chitosan-collagen composite coating at different magnifications.

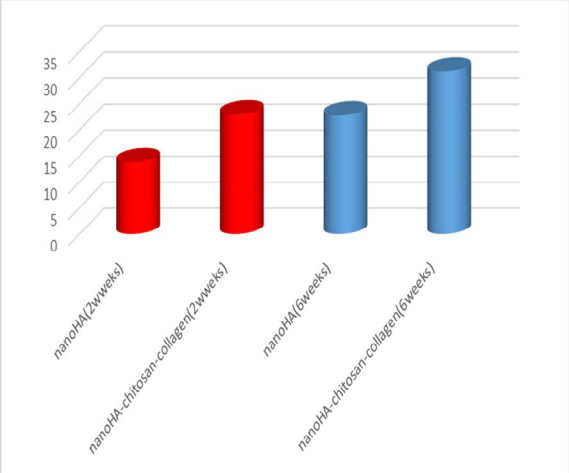
Mechanical Test

Table (1) show the summary statistics of the removal torque value of cpTi coated implants (mean, minimum and maximum values) for both groups (control and experimental groups) for different period of healing. While (figure 7) show a Comparison between the means of coating material at 2 periods of healing.

After two weeks of healing period, the torque value that needed to remove implants coated with nano hydroxyapatite, chitosan and collagen composite was higher than the torque value of nano hydroxyapatite coated implants. Mean of removal torque values of implants coated with nanoHA was (13.76 N.cm), while removal torque mean of composite coating implants was (22.945N.cm). After six weeks of implantation there was increase in removal torque values for both groups, the mean of removal torque values of nano HA coated implants was (22.67 N.cm),and removal torque mean of composite coating group was (31.18N.cm).

Table (1): Summery statistic of removal torque mean (N.cm) of groups for both periods

Group	No	Mean	Min.	Max.
NanoHA (2weeks)	6	13.76	10.59	17.65
NanoHA-chitosan-collagen (2weeks)	6	22.945	17.56	28.24
NanoHA (6weeks)	6	22.67	17.65	28.24
NanoHA-chitosan-collagen (6weeks)	6	31.18	28.24	35.30



(Figure 7): Bar chart of summery of differences in the removal torque means values between all groups.

The equality of means between all groups of implant tested were analyzed by ANOVA Table. This test demonstrated a highly significance difference for both groups at different period of healing (2 and 6weeks), as in table (2).

Table (2): Equality of removal torque mean of all tested groups after 2 and 6 weeks of implantation by ANOVA test

	Sum of squares	df	Mean square	F	Sig.
Between groups	914.083	3	304.694	22.781	.000
Within groups	267.501	20	13.375		
total	1181.584	23			

- P ≤ 0.05 Significant
- P ≤ 0.01 Highly Significant
- P > 0.05 Non Significant

For multiple comparison, the least significant difference (LSD) test used for equality of torque mean values among different groups after 2and 6weeks healing periods. Tab.(3) showed a highly significant difference between groups except nano HA, chitosan and collagen composite coating group at two weeks compared with nano HA coating group at 6wweks of healing period.

Table (3): multiple comparison (LSD) among all groups of different periods of healing

Group		Mean Difference	Sig.
Nano HA (2weeks)	**NanoHA +chi.+C. (2weeks)**	**-9.178**	**.000**
	Nano HA (6weeks)	**-8.001**	**.001**
	NanoHA +chi.+C. (6weeks)	**-17.415**	**.000**
NanoHA +chi.+C. (2weeks	**Nano HA (6weeks)**	**1.176**	**.57**
	NanoHA +chi.+C. (6weeks	**-8.23**	**0.001**
Nano HA (6weeks)	**NanoHA +chi.+C. (6weeks)**	**-9.413**	**0.000**

- P ≤ 0.05 significant
- P ≤ 0.01 Highly significant
- P > 0.05 non significant

DISCUSSIONS

Many studies have concentrated on surface characteristics and chemical composition to control bone healing around dental implants [16]. Dip-coating is an alternative method for prosthetic devices used in orthopedics, it offers a number of advantages over other coating methods such as flexibility, control of coating morphology, chemistry and structure [17]. Hydroxyapatite demonstrates the best bioactivity amongst all the forms of calcium phosphate [18] Chitosan has a set of many characteristic which makes it an excellent choice to be used in tissue regeneration purposes [19]. It has greater effect on biological functions of a cell (cell survival, proliferation and differentiation), helps in healing of damaged bones or blood vessels and maintains structural integrity [20].

FTIR Analysis

The result of FTIR spectra for nanoHA and mixture of nanoHA-chitosan-collagen recorded changes as the shifting of some vibration peaks and change in appearance and intensity. This could be due to molecular interaction between the end group or functional groups of collagen, nanoHA and chitosan [21]. This interaction explain the difference in mechanical force required to remove screws from bone.

Scanning Electron Microscope (SEM)

SEM images of nanohyroxyapatite show uniform, homogenous and without cracking coating over Ti substrate with nanoflower aggregation of particles and nano particle about 200nm. While SEM of nanohyroxyapatite, chitosan and collagen composite coating show uniform coating, crack free and aggregation of particles without formation of phase separation. This mean that organic and inorganic material was mixed well and the inorganic particles is too small this agreed (regardless the difference in material and techniques used) with result of Wang [22].

Mechanical Test

The mean torque value (N.cm) of nanoHA-chitosan-collagen composite was higher than torque value of nanoHA only, it could be rapid new bone formation around implanted screw. The force required to unscrew the implants has been related with the amount of bone in contact with the implant, many studies stated that the changes in the biomechanical features of the implant surface can influence on bone healing and remodeling process [23]. The removal torque values was used in present study as a method to detect the presence of osseointegration at bone-implant contact. Due to bone remodeling and gradual bone formation at bone-implant interface, the present study show that there was an increase in the removal torque value with time and this agreed with Clokie and Bell [24].

CONCLUSION

In conclusion; the rabbits can normally tolerate the coating materials and that showed by the absent of any infection. Higher torque removal mean values for the nano HA,chitosan and collagen composite coating compared to nanoHA coated implants at two implantation periods and this values increased with time for both coating groups.

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