

Histological Assessment of Nano Hydroxyapatite, Chitosan and Collagen Composite Coating on Commercially Pure Dental Implants in Comparison with Nanohydroxyapatite Coating

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Abstract: After high success rate of using dental implants in restoration of missing teeth, attention now shifted toward immediate loading of dental implant through improving the osseointegration, this can be achieved by coating the dental implants by biocompatible materials. The aim of this study was evaluation the osseointegration of dental implants histologically. Dip coating technique was used to obtain thin homogenous coating on commercially pure titanium screws. Tibia of 6 white New Zealand rabbits was chosen as implantation site, screws divided into 2 groups, first group coated with nano hydroxyapatite, chitosan and collagen composite coating, second group coated with nano hydroxyapatite for 2 and 6 weeks of healing periods. Histological analysis showed increasing new bone formation for the nano hydroxyapatite, chitosan and collagen composite coated implants than nano hydroxyapatite coated implants and over the two periods of time. As conclusion screws coated with nano hydroxyapatite, chitosan and collagen composite was more efficient than nano hydroxyapatite coating

Keywords: osseointegration, Dip coating, hydroxyapatite, chitosan, collagen, composite

1. Introduction

Osseointegration can be defined as "the direct connection of osseous tissue to an inert, alloplastic material without intervening connective tissue"^(1,2).

Success and failure of dental implants depend on osseointegration because it is responsible for providing a stable bone-implant interface in order to support a dental prosthesis and transfer applied forces of occlusion without concentrating stresses at the implant-bone interface. Requirement for osseointegration is vital bone and space between the implant and host tissue must be less than 10 nm without formation of any fibrous tissue⁽³⁾.

Due to the biocompatibility and minimum foreign body reaction of titanium, titanium is widely used in fabrication of dental implants in comparison to other materials^(4,5). At the histological level, The implant surface quality (include physicochemical composition and nano- and micro-topography), have a direct effect on the osseointegration⁽⁶⁾.

CaP materials have the ability to bond directly to bone, while titanium doesn't have this ability, so, altering the surface characteristics of titanium have aimed to increase the bioactivity of titanium⁽⁷⁾. Calcium phosphate coatings enhance bone healing and deposition, resulting in rapid fixation of implants⁽⁸⁾.

Collagen and chitosan are polymer, the characteristic feature of polymer is the compatibility with human tissue⁽⁹⁾.

Collagen is the main component of human body, it is responsible for tensile strength and flexibility of bone⁽¹⁰⁾.

Chitosan has been widely used in bone tissue engineering due to its biocompatibility and has the ability to enhance growth of osteoblasts in culture without inflammatory reactions or toxic degradation⁽¹¹⁾. Aim of this study is histological evaluation of nano HA, chitosan and collagen composite as a coating material

2. Material and Methods

Materials

- Commercially pure titanium rod (CpTi) rod grade (Orotig Srl EU company, Italy). - Hydroxyapatite powder (Particles size 98.5%, <40nm), sky spring nanomaterial, USA.)
- Chitosan powder (degree of deacetylation 85%) (Xian Lyphar Biotechnology Co. china).
- Collagen (C5608, Sigma Aldrich, USA)
- Ethanol absolute 99.8%. (Sigma Aldrich, Germany).
- P₂O₅ (Emphos PS-21A, Witco). phosphorous pentoxide, (as thickening material)
- Argon gas (Iraq).
- Acetic acid 2% (Scharlau S.L., Spain).
- Optical microscope (Olympus /542037, Japan).
- Prosthetic engine having straight hand piece (Marathon Saeyang Microtech, Korea)
- Canada balsam (Batch NO. 10862501, European Union).
- Ethanol alcohol 96%.
- Formaldehyde 37%.
- Formic acid 10% (Batch NO. 28380, England).

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- Hematoxylen and eosin (H&E) (Dako, U.S.A).
- Microscopic glass slides and covers (China).
- Paraffin wax (Hinweis, U.K.D).

Methods

After machining of Commercially pure titanium rod into 18 screw shape implants with 3.0 mm in diameter and 8mm in length (threaded part is 5mm and smooth part is 3mm) and pitch height is 1mm .

Cleaning and removing of the oxide layer from the surface of screw shape implants was done by using solution consists of 30% of HNO₃ and 10 %of HF in distilled water then screws were washed by distilled water. After that screws were put in ethanol for 15 minute in ultrasonic cleaner bath, washed by distilled water and dried by oven 100° c for 15 minutes.

Screws divided into two groups, first group coated with nano hydroxyapatite by dip coating, while second group coated with nano hydroxyapatite-chitosan-collagen composite by dip coating. The nano hydroxyapatite suspension involve dissolution of P₂O₅ in 50 ml of absolute alcohol (ethanol) with continuous mixing, Then (7g) of nano-hydroxyapatite powder was added to the solution⁽¹²⁾, second solution prepared by dissolving of collagen in chitosan solution(chitosan solution prepared by dissolving 0.5g of chitosan in 50ml of 2% acetic acid)then mixed with nano hydroxyapatite solution(4g of nanoHA in 50 ml of ethanol). First group sintered at 400°C.while second group coated sintered at 100°C.

Surgical Procedure

Six healthy adult male New Zealand rabbits, , weighing 2 - 2.5 kg, about 11 months age were used. X- Ray was taken prior to surgery (figure 1) to ensure absence of inflammation and adequate bone present for placing the implants. Atraumatic surgery was performed to place the implants in holes in the rabbit tibia, nanohydroxyapatite coated implants placed in hole in right tibia while nano hydroxyapatite ,chitosan and collagen composite coated dental implant placed in hole in left tibia,scarification of the animals was done after 2&6weeks.

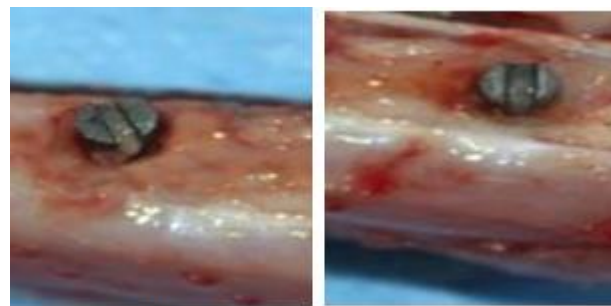


Figure 1: X-ray radiograph prior surgery

3. Results

At the day of sacrifice all implants could not be moved with manual force and were found to stable in its place in the bone, without gross inflammation in the bone, Implants were surrounded by well-developed bone tissue for both coated implant after two and six weeks of healing intervals as illustrated in as shown in (figure 2&3).

Radiographic evaluation have shown that there were no areas of radiolucency between the implant and adjacent cortical bone (figure 4).



(A) Right tibia (B) left tibia
Figure 2: Surgical site after 2 weeks



(A) Right tibia (B) left tibia
Figure 3: Surgical site after 6 weeks



(A) Right tibia (B) Left tibia
Figure 4: X- ray radiograph after 6 weeks

4. Histological Testing

From both 2nd and 6th weeks healing periods three rabbit anesthetized by overdose anesthetic solution and then scarified for histological examination by optical microscope

4.1 Histological features of implant after 2 and 6 weeks healing periods

1) Two weeks after implantation

a) Nano HA coated cp Ti implants

Histological view of nano HA coated screw implanted in rabbit tibia after 2 weeks illustrates border area filled with fibrous connective tissue, fibroblasts, new blood vessels and osteoprogenitor cells (figure 5) indicating delay in bone formation

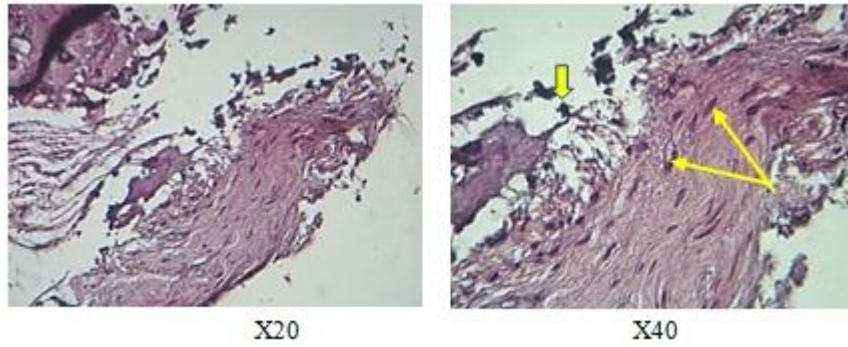
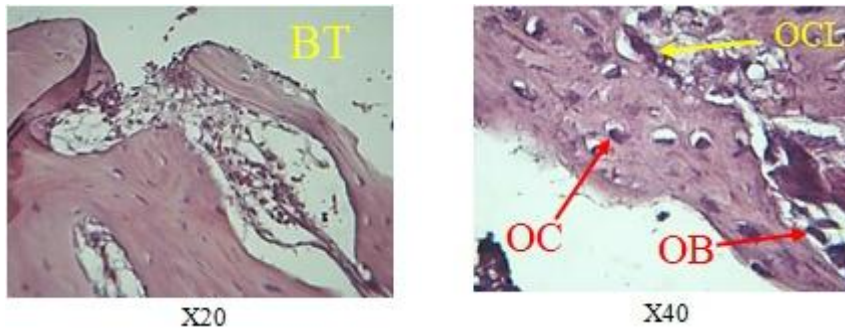


Figure 5: Histological view shows fibrous connective tissue. Fibroblasts (Arrows), and osteoprogenitor cells (arrow head) H&E stain X20&X40

b) Nano HA, Chitosan and Collagen mixture coated cp Ti

Histological view of nanoHA, chitosan and collagen mixture coated screws after two weeks of implantation, shows thread area filled with new bone trabeculae which filled with large

size osteocytes surrounded by osteoblast and osteoclast cells. (Figure 6). Indicating active bone formation



(Figure 6): Histological View Shows New Bone Trabeculae Filled With Osteocytes (OC) And surrounded by osteoblasts (OB) and osteoclasts (OCL), H&E stain X20& X40

2) Six weeks after implantation:

a) Nano HA coated cp Ti

The histological view of nanoHA coated cp Ti screws implanted in the rabbit tibia after six weeks shows the thread site with filled new bone, which filled with osteocyte cells and osteoblast lining the Haversian canal (figure 7).

and lined by osteoblasts (figure 8). With indication of acceleration in bone formation.

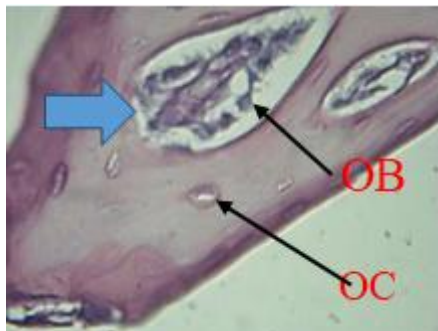


Figure 7: Histological view shows new bone filled with osteocytes (OC) and Haversian canal (Blue arrow) lined by osteoblasts (OB), H&E stain X40

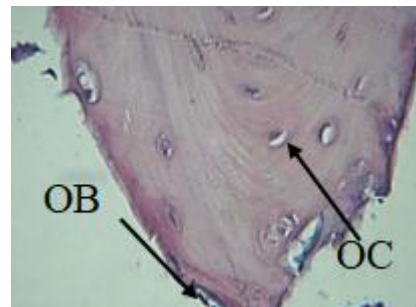


Figure 8: Histological view of previous picture shows mature bone filled with lamellar arrangement of osteocytes (OC) and lined by osteoblasts (OB) reversal line (black arrow), H&E stain X40.

b) Nano HA, Chitosan and Collagen coated cp Ti

The histological view of nanoHA, chitosan & collagen mixture coated cp Ti screws after six weeks of implantation shows formation of mature bone at the thread area. The mature bone filled with lamellar arrangement of osteocytes

5. Discussion

Due to Excellent biocompatibility and lack of toxicity of commercially pure (cpTi) titanium so it is the material most often used in implant manufacturing. Many investigations are being used in an effort to gain desired outcomes at the bone-implant interface⁽¹³⁾. Many studies have focused on surface properties and chemical composition as a way to control bone healing around dental implants⁽¹⁴⁾. The advantage of dip coating was ability of producing multiple thin coatings about 1000 nm thick with a high degree of

uniformity if suitable additives are chosen as P2O5 (P2O5 added to the suspension as a thickening material) and reduction in sintering temperature is also possible⁽¹⁵⁾.

The radiographic examination in this study, showed a direct contact between bone and implant, without radiolucent zones or any abnormal reaction to the implant. However, the absence of such zones is not mean osseointegration, since it is difficult for a clinician to detect changes in the radiographic bone loss at 0.1mm resolution, Histological analysis is the most reliable method to assess stability of implants, and can be performed at any time of implantation⁽¹⁶⁾.

During progressing of the implantation time, a part of the bone appeared on the surface of the implant. This indicated that the improvement in contact between the implant and bone with time resulting in better mechanical properties of bone implant interface. This agreed with Jayaramon et al,⁽¹⁷⁾.

The results of present study showed that placing of coated implants within living bone resulted in osseointegrated implants under typical biological environment for bone formation, the rigidity of connection between osseointegrated implant and the bone and maturation of bone increases with time. This result agrees with Liu et al. 7 Waheed 2013^(18&19).

Regardless of the type of coating material and the duration of the implantation the results gained revealed that no inflammatory reaction had happened during the experimental periods. This is in agreement with the results of Mano et al., 2002 and Lins et al., 2003^(20,21).

6. Conclusion

In this study coating with nano HA, chitosan and collagen composite coating on dental implants showed better results than nano HA coating implants. It shows rapid bone formation and maturation at both period of healing.

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