

The effect of plasma surface treatment on the bioactivity of titanium implant materials (*in vitro*)

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Abstract

Background: The surface of an implantable biomaterial plays a very important role in determining the biocompatibility, osteoinduction, and osteointegration of implants because it is in intimate contact with the host bone and soft tissues. **Objective:** This study was aimed to assess the effect of plasma surface treatment on the bioactivity of titanium alloy (Ti-6Al-4V). **Materials and Methods:** Fifteen titanium alloy samples were used in this study. The samples were divided into three groups (with five samples in each group). Five samples were kept untreated and served as control (group A). Another five plasma samples were sprayed for nitrogen ion implantation on their surfaces (group B) and the last five samples were pre-etched with acid before plasma treatment (group C). All the investigated samples were immersed for 7 days in Hank's balanced salt solution (HBSS) which was used as a simulating body fluid (SBF) at pH 7.4 and 37°C. HBSS was renewed every 3 days. The different surfaces were characterized by X-ray diffraction (XRD), Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM), Energy-dispersive X-ray spectroscopy (EDXA), and Fourier Transformation Infrared Spectroscopy (FTIR). **Results:** Nitriding of Ti-alloy samples via plasma nitrogen ion implantation increased the bioactivity of titanium. Moreover, the surface topography affected the chemical structure of the formed apatite. Increasing the surface roughness enhanced the bioactivity of the implant material. **Conclusions:** Nitridation can be exploited as an effective way to promote the formation of bone-like material on the implant surface.

Key words: Bioactivity, nitridation, nitrogen ion implantation, plasma, plasma surface treatment, surface roughness, surface treatment

INTRODUCTION

Titanium and titanium alloys are widely used in dental implants for their adequate yield strength, hardness, and relatively low weight, high biocompatibility, and excellent resistance to corrosion.^[1]

Many efforts directed toward the design, synthesis, and manufacturing of biomaterials and their medical devices emphasize that they have good mechanical properties, long life, and proper function. Proper functioning of biomaterials and their medical devices are dependent on the bulk structure of the materials.^[2]

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On the other side, biological host responses to biomaterials are influenced by their surface chemistry and texture. Therefore, surface engineering and characterization are critical in the manufacture of biomaterials.^[3]

Bioactivity is the main requirement for a biomaterial to function in a proper manner in a bony site. It includes good biocompatibility favoring bone-like apatite formation, appropriate mechanical properties, and the ability to ensure good skeletal functions,^[4] i.e., proper biointegration.

Bioactivity can be induced on the surfaces of non-bioactive materials either by forming thin ceramic phases like oxides that have the ability to form the functional groups or by the formation of functional groups directly that are able to induce bone-like apatite formation on exposure to a body fluid.^[5]

Bioactive materials have the ability to form an apatite layer on their surfaces in the body and bond to living bone through this bone-like apatite layer. Bone-like apatite is hydroxyapatite with a low crystallinity index. The formation of bone-like apatite layer coating on the surface of substrates is expected to be a useful technique to induce bioactive substrates, which can be done by immersion of the substrates in a solution simulating the body fluid when some functional groups are formed on the substrate surfaces.^[6]

Plasma immersion ion implantation (PIII) has been considered as an effective method to improve the materials' surface-related properties such as hardness, corrosion resistance, and wear resistance.^[7]

Modification of the substrate by plasma is advantageous over other methods through its ability of affect the surface selectively and, thus, the bulk composition of material remains unchanged.^[8]

The plasma immersion ion implantation and deposition (PIII and D) technique is now considered as an attractive method to improve the mechanical properties of the material and its physical and chemical properties also, which necessarily affect the function of a biomaterial once it is placed in a biological environment.^[9]

Nitrogen ions diffuse in titanium and its alloys along the interstitial sites within the cation matrix, leading to formation of a new phase that firmly correlates with the basic structure of the material

and leads to the formation of a compound layer.^[10] Biomedical applications of ceramic coatings of titanium nitride (TiN) were studied due to their good biocompatibility,^[11] excellent corrosion resistance and wear resistance properties.^[12]

Piscanec *et al.* studied the growth of calcium phosphate phase on titanium implants that were coated with TiN. This TiN layer is negatively charged and localized on the substrate surface after surface treatment. So, it enhances the deposition of positively charged Ca ions. Therefore, this TiN coating is convenient for bone-like apatite formation under *in vivo* conditions.^[13]

Biointegration of dental implants is a prime request for their stability and performance. The normal tissue/biomaterial interface is a preliminary intended to realize this request.^[14-16] Hence, it is a significant point of research to attempt modification of the implant surface using various approaches to attain the necessary bioactivity.

This study was aimed to investigate the effect of plasma surface treatment on the bioactivity of titanium alloy (Ti-6Al-4V) and its ability to form calcium phosphate apatite crystals on their surfaces after immersion in a simulated body fluid.

MATERIALS AND METHODS

This study was performed in the Department of Biomaterials, Faculty of Dental Medicine, Al-Azhar University, Egypt. Ethical approval was taken from Al-Azhar University (under number 207/2009).

A total of 15 samples made of Ti-6Al-4V alloy [ASTM International, 100 Barr Harbor Dr, West Conshohocken, PA 19428, United States] were used in this study. The samples were rectangular in shape with dimensions of 15 mm in length, 7 mm in width, and 2 mm in thickness. The samples were fine polished using emery paper starting from grit #400 to #1200, cleaned ultrasonically in deionized water for 5 min, and then dried by an air drier.

The 15 samples were classified into three groups consisting of five samples each, according to the surface treatment received. Five samples were kept untreated and served as control (group A). The surface treatment involved nitrogen ion implantation via plasma spray technique either preceded by acid etching (group C) or not (group B).

The samples of both groups B and C were plasma sprayed for nitrogen ion implantation on their surfaces. The samples of group C were pre-etched using a mixture of 80 ml/l HNO₃, 60 ml/l HF, and 150 ml/l H₂O₂ for 5 min at room temperature.

The plasma focus device was energized by a condenser bank of 30 μF capacitance, 27 nH inductance, and a maximum potential of about 15 kV (3.3 kJ). The condenser bank was charged to the applied voltage using a variable high-voltage charger DC power supply. The electrodes system consisted of a 16-cm-long hollow copper tube of 1.9 cm diameter as anode (central electrode) surrounded by six 1-cm-thick copper rods of length 16 cm forming the cathode. The electrodes system was enclosed in a stainless steel vacuum chamber of 16 cm diameter and 35 cm length. The vacuum was provided by a single-stage rotary pump recharged at an ultimate base pressure of about 0.005 Torr. The substrate holder holding the samples was positioned in the vacuum chamber facing the rim of the anode.

Nitrogen (N₂) gas was used as working gas and the condenser bank was charged up to a potential difference of 12 kV. The condenser bank potential difference was transformed to the plasma focus tube through the air spark gap. In this state, the plasma focus was formed due to dissociation into ions and electron beams; the energetic nitrogen ion beam took the shape of a fountain and spread upward to bombard the facing samples. The process was repeated for 15 times to adequately treat the surface of the sample.

The surfaces of each group were characterized using X-ray diffraction (XRD) (PANalytical's headquarters are in Almelo, The Netherlands), Scanning Electron Microscopy (SEM) (Jeol JSM 5410; Jeol Technic Co., Tokyo, Japan), and Atomic Force Microscopy (AFM) (Thermomicroscopes Autoprobe CP research, scanner 100 m, San Diego, Calif., USA).

The samples were rinsed with distilled water, washed further with ethanol in an ultrasonic bath for 20 min, and then dried in air.

All the investigated samples were immersed for 7 days in Hank's balanced salt solution (HBSS) which was used as simulating body fluid (SBF) at pH 7.4 and 37°C. HBSS was renewed every 3 days to maintain the level of ion concentration allowing for biomimetic coat precipitation on the samples surfaces. After the immersion of the Ti-alloy samples in HBSS, the specimens were removed from the solution, gently rinsed with distilled water, and then dried at 60°C for 24 h.

To assess the biomimetic coat on the differently treated samples, microstructural/morphological changes of the specimens' surfaces were characterized using SEM. Energy-dispersive X-ray spectroscopy (EDXA) (Oxford Instruments, Buckinghamshire, England). The device is attached to JEOL-5400 scanning microscope assembled on the SEM and was used to determine the atomic % of calcium and phosphorous elements, and the Ca/P ratio of the precipitation. Fourier Transformation Infrared Spectroscopy (FTIR) (FT/6300 type A, Jasco, Tokyo, Japan) was used to analyze qualitatively the chemical structure of biomimetic coat which precipitated on the immersed surface.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test at the significance level of $\alpha = 0.05$.

RESULTS

The XRD analysis of the both plasma-treated groups (B and C) revealed the presence of TiN on their surfaces which confirms nitrogen ion implantation in the titanium alloy samples [Figure 1].

The atomic force microscopy (AFM) of group C showed the highest surface roughness among all the investigated groups with more uniform regularities that are attributed mainly to the effect of etchant in 3D image; group C was followed by group B and the control group in this regard (group A) ($P < 0.05$). The 3D images of both groups A and B revealed the similarity of their surfaces suggesting that nitrogen ion implantation has no influential effect on surface roughness [Figure 2].

The SEM of the control group showed polishing marks on the surface of the titanium alloy sample while groups B and C exhibited homogeneous microstructure of

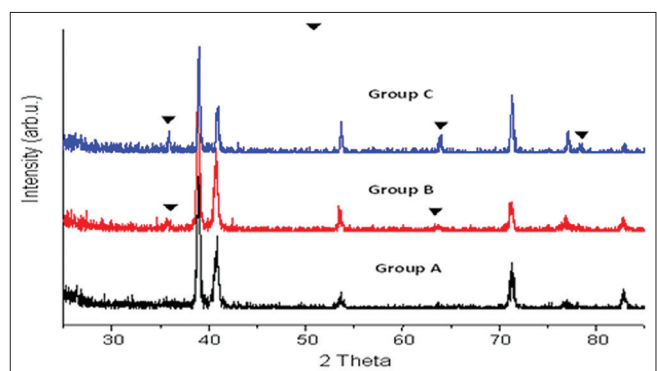


Figure 1: X-ray diffraction pattern of all investigated groups

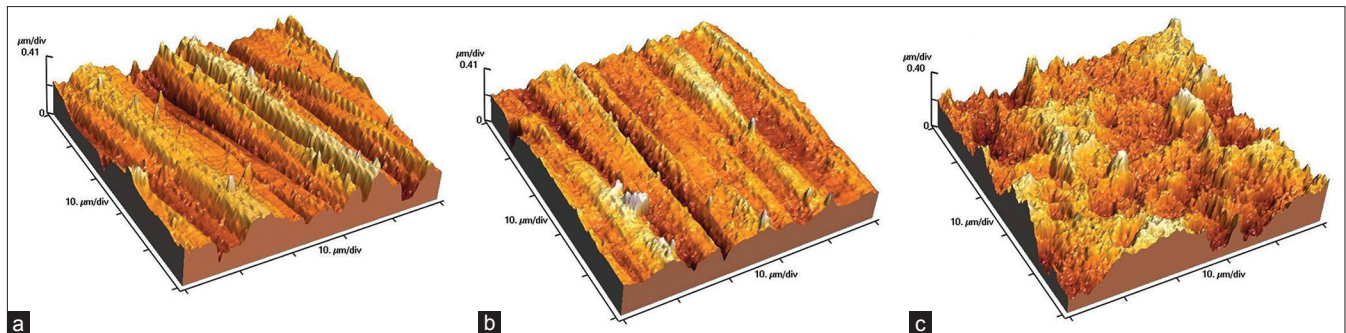


Figure 2: AF micrographs (3D) of the investigated groups a, b and c. (a) Control group. (b) Plasma treated group. (c) Plasma treated with acid etch group

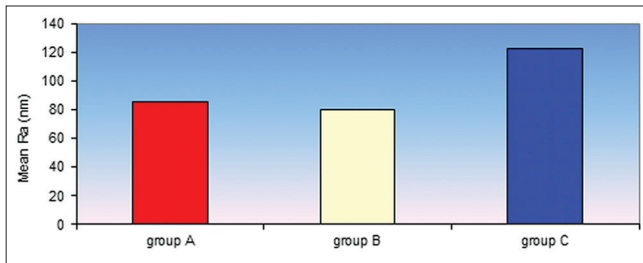


Figure 3: Mean values of surface roughness (Ra) of the investigated groups

titanium nitride layer and the presence of nanospheres that might indicate the implanted nitrogen particles. In both plasma nitrogen ion implantation groups, there were no prominent marks of polishing, distinct irregularities, or even obvious porosities [Figure 3].

After immersion in Hank's solution, the SEM showed discrete small apatite crystals formed on the surface of the control samples and the EDXA spectrum showed phosphorus atomic % of 53.94 and calcium atomic % of 46.05 and the ratio of Ca/P was 0.85, which is much lower than the stoichiometric value of hydroxyapatite (1.67). It indicates the synthesis of monocalcium phosphate monohydrate (MCPM) with the chemical composition $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ and/or dicalcium phosphate dihydrate (brushite) (DCPD) with the chemical composition $\text{Ca}(\text{HPO}_4)_2 \cdot 2\text{H}_2\text{O}$. The SEM of group B showed a sponge-like structure that was obviously seen with considerable thickness. The particles are agglomeration of nanosized particles covering mostly the substrate, and the EDXA spectrum showed phosphorus atomic % of 36.73 and calcium atomic % of 63.26 and the ratio of Ca/P was 1.72. This indicates the formation of, bone like crystals. A (1.72) is rich in calcium than the stoichiometric hydroxyapatite (HA), suggesting improved crystallinity of the particles. The SEM of group C showed spherical globules that were coalesced together in multilayered structure and the EDXA spectrum showed phosphorus

atomic % of 29.85 and calcium atomic % of 70.15 and Ca/P ratio was 2.35, which indicates the formation of calcium-rich amorphous calcium phosphate (ACP) with the chemical formula $\text{Ca}_3(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ [Figures 4 and 5].

After immersion in Hank's solution, the FTIR spectroscopy provided the characteristic absorption bands of functional groups. These functional groups could be evaluated qualitatively to characterize the distribution of mineral content, phosphate content, and carbonate content at $\sim 10 \mu\text{m}$ spatial resolution.

The FTIR analysis of control group (group A) is shown in Figure 6. The spectrum reveals the presence of vibration band at 606 cm^{-1} which represents symmetric stretching vibration of PO_4^{3-} (ν_4). The bands at 865 and at 985 cm^{-1} are for symmetric stretching vibration of PO_4^{3-} (ν_1), while the band at 1019 cm^{-1} is for asymmetric stretching vibrations of PO_4^{3-} (ν_3). The symmetric stretching vibrations of PO_4^{3-} (ν_2) are represented by bands at 1058 and 1090 cm^{-1} , while the band at 1075 cm^{-1} is absent. The symmetric stretching vibration bands of HPO_4^{2-} group are observed at 1113 , 1141 , and 1226 cm^{-1} . The symmetric stretching vibration bands of CO_3^{2-} are captured at 873 , 1414 , and 1453 cm^{-1} . The reflectance bands characteristic of H_2O are observed at 625 , 3083 , 3249 , 3333 , and 3546 cm^{-1} , which denotes the OH^- group of HA. The carbonyl group $\text{C}=\text{O}$ is identified from the bands at 1698 and 1712 cm^{-1} .

The FTIR spectrum of group C reveals the presence of vibration bands at 566 and 605 cm^{-1} , which denote symmetric stretching vibration of PO_4^{3-} (ν_4). The bands at 861 and 981 cm^{-1} are for symmetric stretching vibration of PO_4^{3-} (ν_1). The spectral band at 1020 cm^{-1} which represents asymmetric stretching vibration of PO_4^{3-} (ν_3) is absent. The symmetric stretching vibrations of PO_4^{3-} (ν_2) are represented by bands

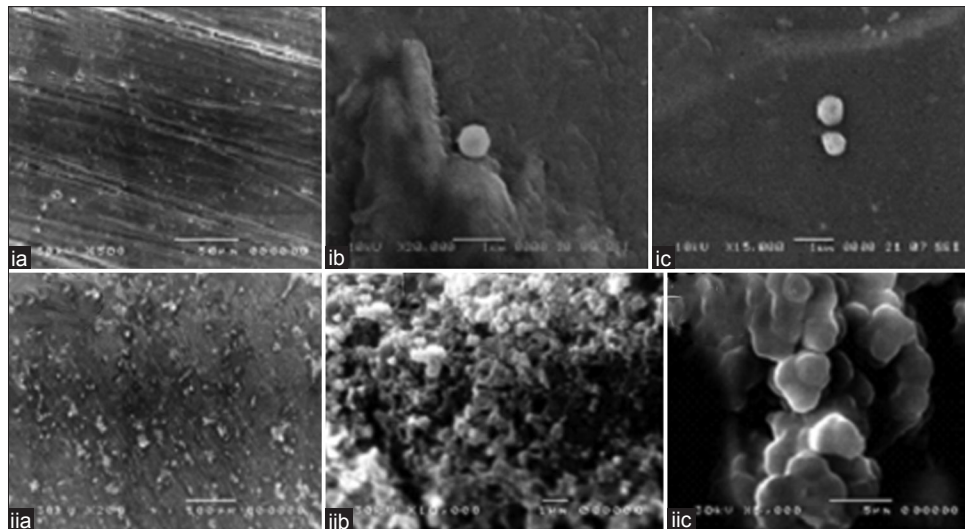


Figure 4: SE micrographs of groups a, b, and c before and after immersion in Hank's solution. (i) Before immersion in Hank's solution. (ii) After immersion in Hank's solution

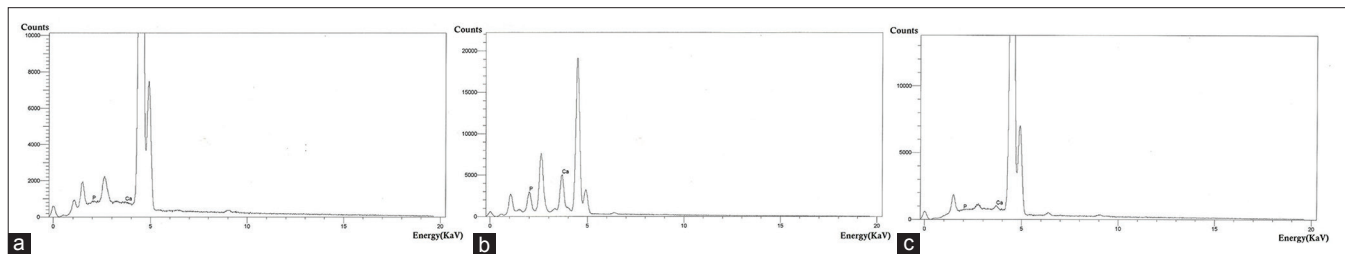


Figure 5: EDX spectrum of the investigated groups a, b and c after immersion in Hank's solution

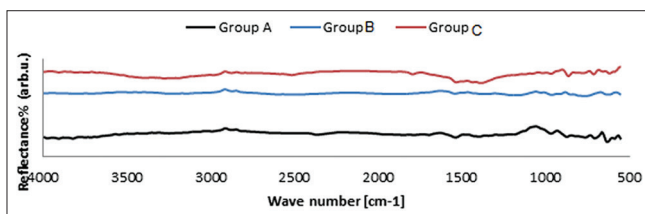


Figure 6: FTIR spectra of the investigated groups A, B and C after immersion in Hank's solution

at 1057 and 1077 cm^{-1} , while the band at 196 cm^{-1} is absent. The symmetric stretching vibration bands for HPO_4^{2-} group are observed at 1115, 1144, and 1224 cm^{-1} . The symmetric stretching vibration bands of CO_3^{2-} are captured at 1416 and 1455 cm^{-1} , while the band at 872 cm^{-1} is absent. The reflectance bands characteristic of H_2O are observed at 625, 3145, 3209, 3429, and 3483 cm^{-1} , which denotes the OH^- group of HA. The carbonyl group $\text{C}=\text{O}$ is identified from the bands at 1712 cm^{-1} .

The FTIR spectrum of group B reveals the presence of vibration band at 606 cm^{-1} which is for symmetric stretching vibration of PO_4^{3-} (ν_4), while the bands at 867, 961, and 985 cm^{-1} are characteristic bands for symmetric stretching vibration of PO_4^{3-} (ν_1). The spectral

band at 1017 cm^{-1} is characteristic for asymmetric stretching vibration of PO_4^{3-} (ν_3). The vibration bands at 1050, 1074, and 1094 cm^{-1} are characteristic for symmetric stretching vibration of PO_4^{3-} (ν_3). The symmetric stretching vibration bands characteristic for HPO_4^{2-} group are observed at 1115, 1143, and 1224 cm^{-1} . The symmetric stretching vibration bands of CO_3^{2-} are captured at 873, 1416, and 1454 cm^{-1} . The absorbance bands characteristic for H_2O are observed at 625, 3030, 3145, 3210, and 3537 cm^{-1} , which denote the OH^- group of HA. The carbonyl group $\text{C}=\text{O}$ is identified from the bands at 1696 cm^{-1} [Figure 6].

DISCUSSION

Titanium and its alloys have many drawbacks such as poor osseointegration because it is a bioinert material and cannot support bone bonding in long-term implants, and induces host inflammatory response at the site of implantation by releasing titanium particles in the surrounding tissues.^[17,18]

In order to serve for a longer period of time without rejection and to solve those medical problems, titanium implant surfaces are coated with specifically engineered

bioactive materials and various elements may be implanted on the surface of biomaterial to improve its bioactivity and stimulate bone formation for proper osteointegration.^[8,19]

Industrially, nitrogen ion implantation via plasma is applied to different alloys such as H13steel and CrNiMo 316 steel as well as Ti6Al4V and CrCoMo, which leads to improvement of wear and friction resistance and enhancement of surface hardness of Ti6Al4V alloy.^[20]

For improving the biological performance of dental and orthopedic implants, many attempts have been made to implant calcium, phosphorus, and magnesium into titanium to enhance the bioactivity of titanium using plasma ion implantation (PIII).^[21-23]

The experimental results obtained by Kokubo *et al.* showed that due to the slow bone growth on titanium surface and delayed fixation of titanium implant to bone that may take several months, bioactivity of titanium surfaces is not high enough. Therefore, many researches were directed toward surface modification of titanium to enhance the interaction of titanium with biological environment and shorten the time needed for proper osteointegration.^[5]

Hank's solution as an SBF is a simplified model that can be used to understand the initial phase of bone-like mineral formation around titanium implant. The SBF is similar to blood plasma in that it is highly saturated with calcium and phosphorous which are needed to apatite precipitation and they have similar ion composition and concentrations in a stable phase.^[24]

The titanium implant is subjected to nitridation and under certain conditions of oxidation forms a very efficient substrate of TiN coating on the implant surfaces which enhances the precipitation of calcium phosphate phase and is exploited as a very effective way to promote the formation of bone-like apatite material on the implant surface. Presence of oxynitride phases on the surface of titanium implants allocates negatively charged surface centers to act as nucleating sites for the adsorption of Ca^{2+} ions from the body fluid.^[13] Nucleation is initiated by the adsorption of Ca^{2+} ions on the substrate followed by a stage of incorporation of negatively charged phosphate groups with formation of an ACP phase.^[25]

The driving force for the deposition of Ca^{2+} ions on passivated titanium surfaces is the electrostatic interaction to negatively charged OH^- groups of the hydrated TiO_2 surface.^[26] Analogously, it was postulated that TiN-coated surfaces behave similarly during the heterogeneous

nucleation of apatite.^[26] However, TiN coatings are commonly assumed to be passivated by a layer of TiO_2 .^[27]

It is well known that surfaces with an isoelectric point (IEP) value much lower than the physiological pH are expected to show a more marked bioactive behavior. Therefore, TiN surfaces that are passivated by a layer of TiO_2 would raise the IEP to display only a moderate bioactivity.^[28] In contrast, Surfaces of TiN are reported to have an IEP value of 4.0,^[29] which suggests an extensive response while in contact with neutral or slightly basic solutions rather than that of TiO_2 surfaces. The presence of mixed phases of oxynitride, which are formed early during oxidation of TiN,^[28] cannot be excluded, and it may be the reason for the low IEP value. Generally, the behavior of TiN films in the biological environment basically depends on their chemical structure and composition.^[30] The partially oxidized hydrated TiN surfaces appear to be able to initiate the spontaneous nucleation of calcium phosphate phases.^[13]

The samples of group C treated by acid etch prior to plasma nitrogen ion implantation had an increased bioactivity as indicated by the increase in Ca/P ratio, compared to the samples of group B which were only treated by plasma nitrogen ion implantation without acid pre-treatment, although the same functional group was present. This clearly indicates that surface roughness along with surface chemistry may have an excellent ability to induce bioactivity of titanium.^[31]

This may be due to increase in surface roughness which, in turn, increases the surface area and/or energy. The increase in surface energy leads to heterogeneous nucleation and growth of HA deposits.^[32]

Moreover, the surface topography might play a role in the chemical structure of the formed apatite. The natural bone apatite has a large, reactive surface, imperfect crystal structure, and non-stoichiometry.^[33]

CONCLUSIONS

The plasma N+ implantation on titanium alloy surfaces permitted the formation of titanium oxynitride. This surface substrate guided the precipitation of HA rich in calcium with a calcium/phosphate ratio ≈ 2.3 and ≈ 1.7 – bone-like crystals, rich in calcium with improved crystal maturity.

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Conflicts of interest

There are no conflicts of interest.

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