SURFACE ANALYSES OF TITANIUM SUBSTRATE MODIFIED BY ANODIZATION AND NANOSCALE Ca-P DEPOSITION

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Statement of problem. Nano-scale calcium-phosphate coating on the anodizing titanium surface using ion beam-assisted deposition (IBAD) has been recently introduced to improve the early osseointegration. However, not much is known about their surface characteristics that have influence on tissue-implant interaction.

Purpose. This study was aimed to investigate microtopography, surface roughness, surface composition, and wettability of the titanium surface modified by the anodic oxidation and calcium phosphate coating using IBAD.

Material and methods. Commercially pure titanium disks were used as substrates. The experiment was composed of four groups. Group MA surfaces represented machined surface. Group AN was anodized surface. Group CaP/AN was anodic oxidized and calcium phosphate coated surfaces. Group SLA surfaces were sandblasted and acid etched surfaces. The prepared titanium discs were examined as follows. The surface morphology of the discs was examined using SEM. The surface roughness was measured by a confocal laser scanning microscope. Phase components were analyzed using thin-film x-ray diffraction. Wettability analyses were performed by contact angle measurement with distilled water, formamide, bromonaphtalene and surface free energy calculation.

Results. (1) The four groups showed specific microtopography respectively. Anodized and calcium phosphate coated specimens showed multiple micropores and tiny homogeneously distributed crystalline particles. (2) The order of surface roughness values were, from the lowest to the highest, machined group, anodized group, anodized and calcium phosphate deposited group, and sandblasted and acid etched group. (3) Anodized and calcium phosphate deposited group was found to have titanium and titanium anatase oxides and exhibited calcium phosphorous crystalline structures. (4) Surface wettability was increased in the order of calcium phosphate deposited group, machined group, anodized group, sandblasted and acid etched group.

Conclusion. After ion beam-assisted deposition on anodized titanium, the microporous structure remained on the surface and many small calcium phosphorous crystals were formed on the porous surface. Nanoscale calcium phosphorous deposition induced roughness on the microporous surface but hydrophobicity was increased.

Key Words
Ion beam-assisted deposition, Calcium phosphate, Anodic oxidation, Surface characteristics, Wettability

Titanium and titanium alloys are widely used for dental and orthopedic implants under load-bearing condition, because of their superior mechanical properties and excellent biocompatibility.[1] Titanium is a reactive metal that forms, spontaneously, in the air, water or any other electrolyte, a thin native oxide film, which is responsible for titanium biocompatibility. This oxide layer is responsible for the bone-bonding characteristics of titanium implants.[2] New research aims are to optimize bone bonding by modifying and improving this passive layer.[3] Among various methods, anodic oxidation (anodization) is reported to be a one interesting method to form thin rough and porous oxide film. The chemical composition, crystallinity, roughness, and topography of the implant surface are changed after the anodizing procedure.[4]

Hydroxyapatite (HA) ceramic has been shown to be biocompatible, nontoxic, and capable of forming a biochemical bond with bone owing to its chemical similarlity to bond mineral, and there have been a number of studies concerning the properties of HA implants.[5] To produce HA coatings on implant materials, a plasma-spraying technique is commonly used. It has been demonstrated that this HA coating on cp Ti implants enhances rapid bone formation because of their improved osteoconductive properties compared with uncoated cp Ti implants.[6] However, problems such as the low adhesive strength of the coating substrate interface and high biodegradation or bioresorption governed by the chemical composition, crystal structure, crystal and grain size, and microporosity have not been resolved.[7,8]

To overcome these problems, thin film coating methods using ion application have been recently developed. Ion beam-assisted deposition (IBAD) has shown favorable effects. Jung et al.[9] investigated that thin HA coating by IBAD method on the as-machined surfaces has shown results comparable to or more favorable than those obtained with a blasted implants. Park et al.[10] suggested the HA coating produced by the IBAD method was also very effective on the aluminum oxide blasted surface, as demonstrated by significantly higher removal torque, bone-to-implant contact, and bone volume than the other group.

Surface properties of an implant play a critical role in the biologic process as bone cells can recognize and respond to surfaces. It has been shown that methods of implant surface preparation can significantly affect the resultant properties of the surface and subsequently the biologic responses and rates of cellular attachment that occur at the surface.[11,12] Recent efforts have shown that the success or failure of dental implants can be related not only to the chemical properties of the implant surface, but also to its micromorphologic nature.[13,14] In addition, cell adhesion to and spreading on a biomaterial are, among other factors, dependent on the surface wettability of the biomaterial.[15] However, not much is known about the optimal surface characteristics of titanium that promote tissue-implant interaction.

In the present study, we applied anodic oxidation and calcium phosphate deposition by IBAD (CaP/AN) to modify the titanium surface. The surface properties of CaP/AN implant materials have not been clarified in detail. Accordingly, we performed surface analysis, surface microstructure, surface roughness, surface chemical structure, and surface wettability. The aims of the present study were to introduce newly developed surface preparation techniques, and to characterize the resultant surface properties on these implant which are expected to improve biomaterial-bone tissue interactions.

MATERIAL AND METHODS

1. Titanium and surface modifications

Titanium substrates were commercially pure titanium discs of 10 mm in diameter and 2 mm in height (Dentium, Seoul, Korea). The discs were prepared to produce four different surface textures as follows;

Group MA: Machined.

Group AN: Anodized surface

Group CaP/AN: Anodic oxidation and calcium phosphate coating.

Group SLA: Sandblasted and acid etched surface.

The discs in group AN were anodized with the pulse power. The electrolyte solution contained calcium acetate [$(CH_3COO)_2CaH_2O$] and calcium glycerolphosphate ($CaC_3H_7O_6P$). In group CaP/AN, thin calcium phosphate coatings were deposited on titanium substrates in an Ion Beam Assisted Deposition (IBAD) system using a sintered hydroxyapatite (HA) target.

2. Surface analysis

All specimens were washed with alcohol and distilled water and sterilized with ethylene dioxide gas. The prepared titanium discs were examined as follows. At the first, the surface morphology of the discs was examined using SEM (JSM-840A, JEOL, Japan). The SEM micrographs were taken at several randomly chosen areas on each specimen ($\times 5000$, $\times 30000$). A confocal laser scanning microscope (CLSM), PASCAL LSM5 (ZEISS, Germany), was used for three dimensional roughness measurements to get more detail topographic characterization form one sample of each preparation. The area of measurement was $450 \times 450 \mu m$. The following parameters were calculated: S_a, arithmetic mean deviation of the surface; S_{max}, maximum peak-to-valley height of the surface. Five readings were made randomly on 4 samples and the results were averaged. And phase components were analyzed using thin-film x-ray diffraction (TF-XRD; X,Pert PRO, PNAalytical. USA).

3. Surface wettability

Wettability analyses were performed by contact angle measurement and surface free energy calculation. Contact angle measurements were performed with a contact angle measuring system from Phoenix 300 (Surface Electro Optics Co., Korea), equipped with a video charge-coupled device (CCD) camera and Image XP ver 5.6 software. Contact angle were measured with distilled water, formamide, bromonaphtalene employing the sessile drop method and subsequently surface free energy were calculated (Table I). Contact angles were obtained on the photographs as follows: $10\mu l$ droplets of each liquid were positioned on test specimens by means of a 1mL syringe with a blunt point. A tangent to the droplet was drawn from the point of air-fluid-solid phase intersection. Contact angles between this tangent line and the test specimen surface were calculated from enlarged photonegatives of the droplets (Fig. 1). Measurements of contact angle

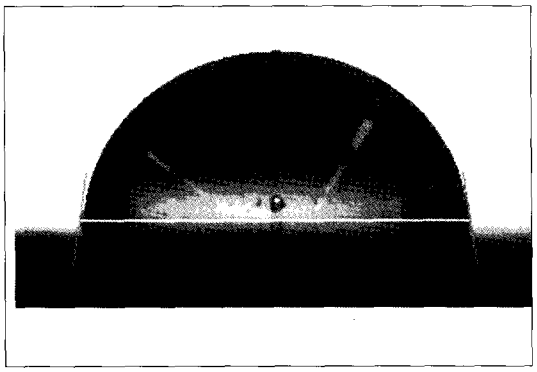

Fig. 1. Contact angle measurement.

Table I. Surface energy components and parameters of test liquids used in contact angle and surface energy determination

mN/m	Distilled water	Formamide	Bromonaphtalene
Tension	72.8	58.2	44.4
Dispersive component	21.8	39.0	44.4
Polar component	51.0	19.2	0
P-acid	25.5	2.3	0
P-base	25.5	39.6	0

were performed five times each test solution at room temperature to provide adequate replications for statistical analysis. Surface free energy was calculated using the Lewis Acid/Base method for 3 liquids.

4. Statistical analysis

Experiments were repeated three to five times. Statistical evaluation of data was performed using the software package SPSS/PC statistics™ 13.0(SPSS Inc., Chicago, IL, USA). Data were expressed as mean values and standard deviation of each group of samples. Statistics were performed by analysis of variance (ANOVA), followed by and Scheffe' s method as a post-hoc comparison. A differences of a $p<0.05$ was considered to be statistically significant.

RESULTS

1. Surface morphology

Figure 2 displays the finding of Scanning electron microscopy (SEM) of disks. The machined disk revealed uniformly smooth surface with typical machining grooves and ridge as produced by manufacturing instruments. The examination of anodized samples showed smooth surface exhibiting multiple micropores. The topography of the specimens in anodized and Ca-P deposited was similar to that in group AN in terms of the number and size of pores. However, tiny homogeneously distributed crystalline particles were shown attached on specimens in group CaP/AN. The SLA surface had a duplex surface morphology of a coarser microstructure and a finer microstructure. Much finer micropits were superimposed onto the rougher indentations or cavities. Micropits of the SLA surfaces had a sharp edge and pyramidal aspect of the protrusions.

2. Surface roughness

Table II showed results of the surface roughness measurements. The roughness parameters of disks showed significant differences in the S_a values of each specimen ($p < 0.05$). The order of surface roughness values were, from the lowest to the highest, machined group, anodized group, anodized and Ca-P deposited group, and sandblasted and acid etched group.

3. X-ray diffraction (XRD)

Figure 3 showed the result of XRD analysis. The TF-XRD patterns of the machined Ti substrate and SLA surface expressed only titanium peaks. The TF-XRD pattern of anodized Ti substrate exhibited anatase and amorphous oxide. The TF-XRD patterns of group CaP/AN sample exhibited calcium phosphorous crystalline structure.

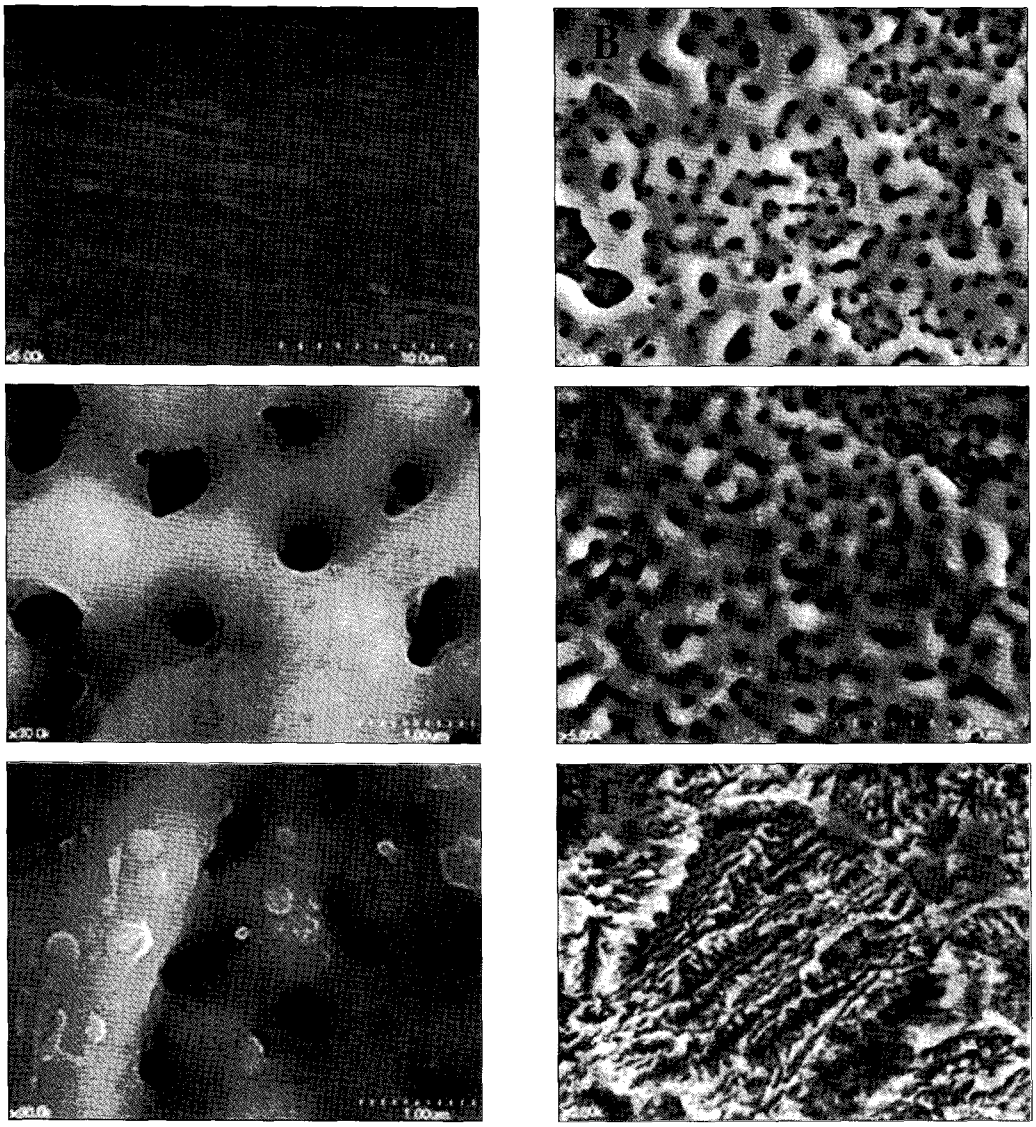

Fig. 2. SEM photographs of each specimen: (A) machined (× 5,000), (B) anodized (× 5,000), (C) anodized (× 30,000), (D) anodized-CaP coated (× 5,000), (E) anodized-CaP coated (× 30,000), and (F) sand-blasted and acid etched surface (× 5,000).

4. Surface contact angle

Table III gives the contact angles obtained by the sessile drop method on the different surfaces. The mean contact angles increased in the order of group SLA, group AN, group MA, and group CaP/AN. There was no significant difference between group AN and group MA for water contact angles, and significant difference between group MA and group CaP/AN for bromonaphtalene values ($p < 0.05$). There were significant differences among four groups for formamide values ($p < 0.05$). In the group SLA, the shape of the drops was highly asymmetric and not stable during the measuring period.

Table II. Surface roughness of the titanium disks

Group	S_a (μm)	S_{max} (μm)
MA	0.297 ± 0.006 [a]	3.071 ± 0.818 [a]
AN	0.549 ± 0.020 [b]	8.031 ± 0.737 [b]
CaP/AN	0.641 ± 0.087 [c]	8.740 ± 0.737 [b]
SLA	1.185 ± 0.033 [d]	14.646 ± 0.541 [c]

Data were expressed as mean values and standard deviations. Statistical significance was tested by ANOVA ($p < 0.05$). The same letters indicate nonsignificant differences between groups on Scheffe's multiple comparison tests.

Table III. Contact angles (degree) of liquid drops on the samples

Groups \ Liquid	Distilled water	Formamide	Bromonaphtalene
SLA	69.35 ± 2.11 [a]	51.36 ± 0.97 [a]	7.26 ± 0.60 [a]
AN	73.18 ± 0.22 [b]	62.60 ± 1.28 [b]	16.23 ± 0.49 [b]
MA	75.33 ± 0.56 [b]	65.63 ± 0.62 [c]	19.07 ± 0.49 [c]
CaP/AN	86.17 ± 0.69 [c]	77.80 ± 1.84 [d]	20.03 ± 0.60 [c]

Data were expressed as mean values and standard deviations. Statistical significance was tested by ANOVA ($p < 0.05$). The same letters indicate nonsignificant differences between groups on Scheffe's multiple comparison tests.

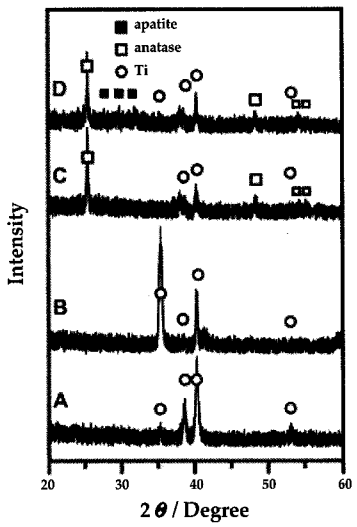

Fig. 3. TF-XRD patterns of each specimen: (A) machined, (B) sandblasted and acid etched surface, (C) anodized, and (D) anodized-CaP coated surfaces.

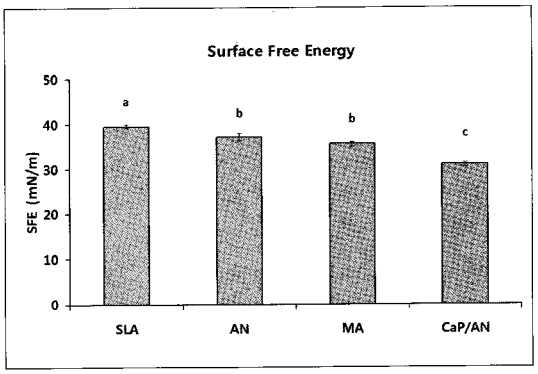

Fig. 4. Surface Free Energy (mN/m) of the Samples: Data were expressed as mean values and standard deviations. Statistical significance was tested by ANOVA ($p < 0.05$). The same letters indicate nonsignificant differences between groups on Scheffe's multiple comparison tests.

5. Surface free energy

Figure 4 showed the results of surface free energy (SFE). Values for surface free energy varied between 30.98 mN/m and 39.59 mN/m decreasing in the order SLA, AN, MA, and CaP/AN. There was no significant difference between group AN and group MA ($p < 0.05$).

DISCUSSION

This study was perform to characterize the surface properties which affect the biologic responses on surface of a new CaP/AN implant materials with a thin calcium phosphorus layer on titanium by means of anodization and ion beam-assisted deposition(IBAD). And their characteristics were compared with those of surfaces that prepared with different roughnesses and surface physicochemical properties. After anodization and IBAD treatment, the porous structure remained on the surface and many small crystals appeared. The crystals were calcium phosphorous, and they were about several hundred nanometers in size, which induced roughness on the microporous surface. The main composition of this oxide film is TiO_2 in anatase and amorphous forms. The contact angles for test liquids on anodized-CaP coated surface were higher than on other surfaces, and its surface free energy was lower than that of cp-Ti.

Cell behavior on biomaterial surfaces depends upon implant cell interactions, correlated with surface properties. Surface chemical composition, roughness, texture, morphology, and hydrophilicity strongly affect cellular responses in contact with the implants.[16-19] Several recent studies have shown that the surface energy of biomaterials strongly influences the initial cell attachment and spreading of osteoblastic cells on the biomaterial surface.[20-27] Hallab et al.[22] thought that surface energy might be a more important determinant of cell adhesion and proliferation, and might be more useful than surface roughness for generating cell adhesion and cell colonization on the engineered tissue scaffolds. Den Braber et al.[23] evaluated the effect of parallel surface microgrooves and surface energy on cell growth. The most significant conclusion was that physicochemical parameters such as wettability and surface free energy influence cell growth but play no measurable role in the shape and orientation of cells on microtextured surfaces. Meyer et al.[24] quantified the rate of cell attachment and revealed a constant dependence on a material within the first 7 h of culture. This may reflect the wettability. After 24 h of incubation, however, measurement of cell attachment revealed less significant differences between the materials. Other research teams observed similar results. Webb et al.[25] have stated that, when material surfaces are exposed to dilute serum, fibroblast attachment and fibroblast spreading are greater on hydrophilic surfaces compared to hydrophobic surfaces. However, differences between the hydrophilic surfaces in water wettability influenced cell attachment but not spreading or cytoskeleton organization. Redey et al.[26] studied osteoclast adhesion and activity on synthetic hydroxyapatite, carbonated hydroxyapatite, and natural calcium carbonate and the relationship to surface free energies. Surface energy was found to play an essential role in osteoclast adhesion, whereas osteoclast spreading was found to depend on surface chemistry, especially on protein adsorption and newly formed apatite layers. Ponsonnet et al.[27] also suggested that fibroblast proliferation cannot be directly correlated to the water contact angles. Roughness (in the micron range) seems to be the major parameter for cell proliferation. Indeed, only smooth surfaces (roughness less than 1 ㎛) showed significant proliferation, whatever the wettability.

In general, it has been hypothesized that an

increase in surface roughness results in an increase in contact angle. It was surprising that the high roughness did not always indicate the high contact angle. Lim et al.[28] confirmed that for cp Ti, the contact angle increased linearly with Ra when contact angle was greater than 45 degrees, while it decreased linearly with Ra when contact angle was less than 45 degrees. In present study, results for only CaP/AN group is agreement with the work of Lim et al. The SLA surfaces revealed highest roughness value among the samples but had a lowest contact angle. This result indicates that the SLA surface was more hydrophilic and exhibited markedly improved wettability. It seems that this parameter is related with the increase specific surface area. And the open microtopography of SLA surface is more likely at the origin of more hydrophilicity, probably due to capillary forces. Because of the anisotropy of the different surface topographies in sandblasted surface, the shape of the drops was highly asymmetric and not stable during the measuring period. It was found that the water contact angle on SLA surface was strongly dependent on surface microtopography and micromorphology.[29,30]

The surface crystallography should also be considered as an important factor at the same time. The contact angle appears to be related to the crystalline structure of the oxide films formed on titanium and it alloys. Lim et al.[28] also suggested that the group in which the contact angle increased linearly with R_a had only a rutile type oxide layer, while the group that decreased linearly with R_a had a mixture of dominant rutile and anatase oxides. In our experiment, the crystalline structure of oxide film didn't seem to affect the surface wettability. AN specimens had only anatase type oxides and CaP/AN specimens had anatase type oxides and calcium phosphorous crystalline structures. The wettability of AN group was not different with machined surface and that of CaP/AN was decreased. The chemical composition and surface chemistry of titanium alloys contribute to these results. Other surface properties should also be considered as of importance for biological response and may be more critical parameters for biocompatibility than surface roughness itself.

Schakenraad et al.[31] concluded poor cell spreading of various cell types on low surface free energy substrata and good cell spreading on high surface free energy substrata. The inflection point of the curves, indicating the change from poor cell spreading to good cell spreading, is around 57 mN/m. This limit is higher than the SFE values obtained in the present study and the curve corresponding to cell proliferation indicates that, before the inflection point in the 31.0-39.6 mN/m range, the relationship between SFE and proliferation can be reversed: proliferation can be higher for lower SFE in CaP/AN group. Webb et al.[25] and Ponsonnet et al.[27] considered that surfaces with a moderate wettability favor a higher cell adhesion behavior.

When dental root implant material is implanted into bone tissue, many biological reactions occur between its interface and the bone. The surfaces of dental root implants are usually in contact with a variety of ions, blood, serum proteins, bone morphogenetic protein, and noncollagenous bone proteins (e.g., bone sialoprotein, osteopontin, osteocalcin) which are present in the tissue fluid in vivo, and adsorption of these proteins is a function of the surface energy of the TiO_2 matrix formed on the titanium surface and calcium phosphorous layer. Furthermore, the chemical or physical state of the surface may affect the adsorption of ions and proteins that support cell attachment.[32] Adsorption of proteins always precedes to cell adhesion, which required for cellular adherence to the implant material surface, and wettability on the surface of the implant material is also thought to be important.[24] Van Oss[33] studied that proteins adsorb at significantly lower

concentrations to hydrophilic than to hydrophobic substrates whereas cells prefer hydrophilic surfaces. Groth and Altankov[34] studied the role of tyrosine phosphorylation during fibroblast spreading on surfaces with different values of wettability. The authors concluded that the effect of hydrophobic substrata on cells with respect to adhesion and proliferation is due to a transfer of signals via integrins from the substratum to the cell interior. The preadsorption of fibronectin on hydrophobic substrata may provide better initial conditions, thus improving the tissue compatibility of the material. In our study, anodization and IBAD treatments resulted in a higher surface hydrophobicity of the original Ti surface. Calcium phosphate ceramics have been shown to have a very high adsorption capacity for serum proteins compared with other materials.[35-39] It is well admitted that the cellular response to a material is only a secondary event and the presence of adsorbed protein layer is essential in mediating cell response to the material.

CONCLUSION

1. Anodized and calcium phosphate coated specimen showed multiple micropores and tiny homogeneously distributed crystalline particles.
2. Calcium phosphate deposition induced roughness on the microporous anodized surface.
3. Anodized and calcium phosphate deposited group was found to have anatase type oxides and exhibited calcium phosphorous crystalline structures.
4. Hydrophobicity was increased in the anodized and calcium phosphate deposited specimen.

Further study of cellular and tissue responses, particularly long-term studies of cell culture or animal studies, would also help elucidate the improved biocompatibility and bioactivity in titanium modified by combining method of the anodization and nano-scale calcium phosphate coating using ion beam-assisted deposition.

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