

Application of solution plasma surface modification technology to the  
formation of thin hydroxyapatite film on titanium implants

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## Introduction

The use of dental implants has revolutionized the current treatment of partially and fully edentulous patients owing to a high level of predictability and a wide variety of treatment options. In 2006, the estimated number of dental titanium implants placed in the United States was over 5 million. A major consideration in designing dental implants is the production of surfaces that promote desirable responses in the cells and tissues.

Hydroxyapatite (HA) coatings on titanium implants facilitate rapid bone formation owing to their excellent osteo-conductive property. HA coated implants have been reported to stimulate bone healing, which enhances improvement in the rate and strength of the initial implant integration. For enhanced implant stability and bio-integration to bone tissue, various methods have been applied to coat the titanium implant with HA. For example, plasma spraying, electrophoretic co-deposition, ion-beam-sputter deposition, dip coating in a simulated body fluid, electrochemical deposition, blast coating, and thermally induced liquid-phase deposition. Among these methods, titanium implant bodies coated with HA in dry processes, such as the plasma spraying method and ion-beam-sputter deposition method, have already been used clinically. The HA coating methods in dry processes, however, cannot be applied to titanium implant bodies with porous surface structures. For coating on the titanium implant with a three-dimensional porous surface structure with HA film, Kuroda and Tamura developed the titanium substrate heating method in a liquid. Since the solubility product of HA decreases with an increase in temperature above 16 °C, they electrically heated the titanium substrate by applying a large current with an AC or DC power source to the substrate in calcium phosphate solutions to precipitate HA crystals on the titanium surface. These two studies

demonstrated that the HA crystals that precipitated were needle-like or plate-like in shape and the formation of a uniform and dense HA film consisting of fine and spherical HA crystals could not be achieved. Another shortcoming of the titanium substrate heating method is that it requires a large electric power source exceeding 1,000 V for the deposition of HA crystals on the dental implant body surface by Joule heating.

The solution plasma technique to coat an implant surface with HA spherical particles is an entirely new technology. To date, this solution plasma technique has been used in the fields of chemistry and applied physics. When a pulsed voltage is applied between the two electrodes, a glow discharge takes place in the liquid, which generates plasma in the gas phase. This plasma produces thermal energy and light along with a reactive chemical species such as hydrogen and hydroxide (OH) ions. With this novel surface modification technique, the temperature of the solution in contact with the titanium surface can easily be increased with the thermal energy generated by the solution plasma. In this case, a thin and uniform HA layer is expected to be formed on a porous surfaced titanium implant, which will induce the rapid bone growth into the pore space of the implant body, and also enhance the biological anchorage of the implant with bone.

The present study was aimed at achieving a thin and uniform HA layer covering the titanium with a porous surface structure by using the solution plasma technology. The mechanism of the HA precipitation on the titanium during the solution plasma treatment was elucidated. The feasibility of this HA film coating method using solution plasma technology to other implant materials, such as zirconia, was also investigated.

#### Materials and methods

## 2.1 Substrate

Commercially pure titanium, porous titanium with 69.7% porosity, and zirconia disks were employed in this study.

Group A – Titanium (Ti) (JIS Type-2, J. Morita corp.); size of the specimen was 14mm in diameter and 2mm in thickness.

Group B – Porous-Ti (powder sintering product with 69.7% porosity, Nagamine manufacturing company); size of the specimen was 14 mm square, 1 mm in thickness, and the pore size was  $287 \pm 87$   $\mu\text{m}$ .

Group C – Zirconia ( $\text{ZrO}_2$ ) [yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) GC corp.], size of the specimen was 14mm in diameter and 2mm in thickness.

The Ti disks and the Y-TZP disks were polished with silicon carbide abrasive papers (#240 and then #600). The polished groups were named as “Polished-Ti” and “Polished-Y-TZP”. “Porous-Ti” was used without polishing for the experiments.

Half of the polished-Ti and porous-Ti substrates were subjected to alkaline treatment prior to the solution plasma treatment (SPT). The disks were immersed in 5 M NaOH solution at 60°C for 24 h.

After alkaline treatment, the polished titanium (Ti) group was named as “Ti-AT” and the porous titanium substrate (Porous-Ti) group was named as “Porous-Ti-AT”.

## 2.2 Preparation of the mineralizing solution

A metastable calcium phosphate solution was used in this study. The composition of the solution is shown in Table 1. The Ca/P molar ratio of the mineralizing solution was 1.67, and it also contained 10mM HEPES-KOH for buffering at pH 7.4. The final ionic strength of this solution was adjusted to 0.16 by adding 150mMKCl. The preparation of the solution was completed by dissolving reagent grade  $\text{CaCl}_2$ ,  $\text{KH}_2\text{PO}_4$ , KCl, and HEPES into deionized distilled water. The degree of supersaturation with respect to hydroxyapatite was  $3.85 \times 10^7$  at 37 °C.

### 2.3 Solution plasma treatment

A solution plasma treatment was performed in approximately 150mL of the mineralizing solution at 29°C. A pulse plasma generator (MPP-NV04, Pekuris) was used and the Polished-Ti and Porous-Ti substrates were placed at a distance of 5 mm from the electrodes within a glass vial (Figure 1). Plasma was generated at a voltage of 5 V and pulse width of 3  $\mu\text{s}$  for 30 min. The mineralizing solution was stirred using a magnetic stirrer (Ion Stir 7d, Central Kagaku Co. Ltd.) at a rotational speed of 240 rpm. The duration of the solution plasma treatment was 30min.

When the solution plasma surface modification technique was applied on “Ti-AT” and on “Porous-Ti-AT”, they were named as “Ti-AT-SPT” and “Porous-Ti-AT-SPT”. Polished yttria-stabilized tetragonal zirconia polycrystals (“Polished-Y-TZP”) were subjected to the solution plasma treatment with the same conditions as titanium. The Polished-Y-TZP after being subjected to solution plasma treatment was termed as “Y-TZP-SPT”.

### 2.4 Characterization of the HA layer formed on various substrates

Morphologic and crystallographic features of deposited films were examined by scanning electron microscopy (SEM) and X-ray diffractometry (XRD). The contact angle of the water droplets (20  $\mu$ L) on the titanium surfaces was measured by a contact angle analyzer after each surface treatment.

#### 2.4.1 SEM observation

The morphology of the titanium surface after SPT and immersion treatments was examined using a scanning electron microscope (SSX-550, Shimadzu Corporation) with an acceleration voltage of 10 kV.

#### 2.4.2 X-ray diffraction

The crystallographic features of the deposited films were examined using an X-ray diffractometer (JDX-3500, JEOL Ltd.) with Cu K $\alpha$  radiation ( $\lambda$ = 0.1541 nm) at room temperature. The  $2\theta$  range was 20°–80°, and the XRD profile was recorded at step-scan intervals of 0.02° at a scanning speed of 8.0°/min at 40 kV and 150 mA.

#### 2.4.3 Measurement of the contact angle of water droplets on the surfaces

To evaluate the wettability of the titanium and zirconia disks before and after surface treatment, the contact angle of water droplets (20  $\mu$ L) on the surfaces was measured using a contact angle analyzer (Phoenix Alpha, SEO, Republic of Korea) at 0h, 1day, 3days, 7days, and 14days after each surface treatment.

### 2.5 Evaluation of cytocompatibility

#### 2.5.1 Cell line

The MC3T3E1 cell line was used in this study to evaluate the cell attachment of the titanium samples before and after SPT.

#### 2.5.2 Initial attachment of cells

There were 6 groups for each experiment, that is, 30-day aged (“Polished-Ti” and “Ti-AT-SPT”), 7-day aged (“Polished-Ti” and “Ti-AT-SPT”) and fresh “Polished-Ti” and fresh “Ti-AT-SPT”. Four specimens from each group were placed on a 24-well plate, disinfected with 1 mL of 70% ethanol for 10 min and washed with sterilized distilled water for 10 min. The number of MC3T3E1 cells was adjusted to be  $5 \times 10^4$  cells/mL in a regular medium consisting of  $\alpha$ -modified minimum essential medium ( $\alpha$ -MEM; with L-glutamine and phenol red, Wako Pure chemical industry Ltd.) supplemented with 10% heat-inactivated fetal bovine serum. Adjusted cells were seeded on a sample with 1 mL per well. After 4 hours of incubation under a humidified atmosphere of 5% CO<sub>2</sub> at 37°C, the cells were detached by trypsin-EDTA and the number of cells was counted by a haemocytometer.

#### 2.5.3 Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA), and the Games-Howell post hoc test with the significance level of ( $p < 0.05$ ).

### Results

#### 3.1 Variation of solution temperature in the vicinity of the sample with time during SPT

The variation of the solution temperature with time during SPT measured by a thermocouple placed in the vicinity of the sample. The temperature increased rapidly and exceeded 60 °C within 3 min after starting SPT, and then increased slowly and reached an almost constant value of 80 °C at 30 min. After 30 min of SPT, crystals were precipitated owing to heterogeneous nucleation, both on the sample surface and in the solution.

### 3.2 Characterization of titanium and zirconia surfaces subjected to SPT and IT in the calcium phosphate solution.

#### 3.2.1 SEM observation

In the polished titanium surface after SPT (Ti-SPT) and after 5 M NaOH treatment and subsequent SPT (Ti-AT-SPT) crystals formed both on the Ti-SPT and the Ti-AT-SPT. Crystals with diameters between 5  $\mu\text{m}$  to 20  $\mu\text{m}$  precipitated sparsely and did not cover the whole surface of the Ti-SPT, while the fine spherical crystals with a diameter of 5  $\mu\text{m}$  precipitated and covered the whole surface of the Ti-AT-SPT.

The SEM photographs of the porous titanium surface after 5 M NaOH treatment and subsequent SPT (Porous-Ti-AT-SPT). Fine spherical HA particles were uniformly precipitated over the entire surface, including the areas recessed in the shape of the porous structure and also the inner surface of the pores.

The zirconia surface after SPT (Y-TZP-SPT) was also observed that uniform and fine particles had precipitated and covered the entire surface without 5 M NaOH pretreatment.



### 3.2.2 Analysis of precipitated particles on Ti-AT-SPT, Ti-AT-IT60°, and Y-TZP-SPT by XRD and EDX

X-ray diffractogram obtained from Ti-AT-SPT and Ti-AT-IT60° for 24 h, together with that of Polished-Ti and the synthetic HA powder. All of the diffraction peaks were assigned to HA or the titanium substrate under the deposited film. For Ti-AT-SPT and Ti-IT60°C, the diffraction peak of the deposited HA crystals at 25.9° (2 $\theta$ ), which corresponded to the (002) lattice plane, was relatively higher than the other diffraction peaks at around 32°, unlike that observed for the synthetic HA powder. This indicated that the spherical HA crystals precipitated on Ti-AT-SPT and Ti-IT60°C were slightly oriented with the c-axis perpendicular to the titanium substrate. These results clearly demonstrated that 5 M NaOH treatment and subsequent SPT for 30 min in the calcium phosphate solution were effective in coating the entire surface of titanium with an HA film composed of fine crystals in a relatively short treatment time.

X-ray diffractogram from the Y-TZP-SPT and Polished-Y-TZP shows no diffraction peaks of the precipitates were observed in the X-ray diffractogram obtained from the Y-TZP-SPT together with that of the Polished Y-TZP and synthetic HA powder, probably owing to the fine size of the particles or low crystallinity of the precipitates on the Y-TZP-SPT.

An energy dispersive X-ray spectrum obtained from the Y-TZP-SPT surface. Unfortunately, the peak for P K $\alpha$  was overlapped with Zr L $\alpha$  although a strong Ca K $\alpha$  peak was observed. The small peaks of Cl K $\alpha$  and K K $\alpha$  were also observed. These results did not reveal the presence of HA; however, the particles composed of some calcium phosphate were confirmed to be precipitated on the Y-TZP-SPT surface.

### 3.3 Crystals on the titanium surfaces after immersion in the calcium phosphate solution

The polished titanium after immersion in the calcium phosphate solution at 37 °C (Ti-IT37°) and after 5 M NaOH treatment and subsequent immersion in the calcium phosphate solution at 37 °C (Ti-AT-IT37°). No crystals were deposited on the Ti-IT37° for up to 7-days immersion. In contrast, spherical particles consisting of plate-like HA crystals were deposited on part of the Ti-AT-IT37° after 1-day immersion. These crystals grew with time and they covered almost all of the surface of the titanium after 7-days immersion. These results were consistent with those reported previously.

The SEM photographs of the polished titanium after immersion in the calcium phosphate solution at 60 °C (Ti-IT60°) and after 5 M NaOH treatment and subsequent immersion in the calcium phosphate solution at 60 °C (Ti-AT-IT60°). No spherical particle precipitation was observed on the Ti-IT60° after immersion in the solution for 30 min. Precipitated crystals between 5 µm and 40 µm in diameter were found on the Ti-IT60° after immersion for 6 h, and these HA spherical particles grew to the large size spherical particles between 40 µm and 100 µm in diameter after immersion for 24 h. On the Ti-AT-IT60°, small crystals precipitated after immersion for 30 min. The density of the crystals increased owing to the growth of crystals with time. It was observed that the crystal formation and its growth rate were markedly enhanced with 5 M NaOH treatment and also with an increase in the temperature of the calcium phosphate solution.

### 3.4 Change in the contact angle of water droplets on Polished-Ti and Ti-AT-SPT with aging time

Changes in the contact angle of 20  $\mu$ L water droplets observed on Ti, Ti-AT, and Ti-AT-SPT with aging time, the contact angle value for Ti increased as the Ti disk aged, suggesting that the surface property changed from being hydrophilic to hydrophobic. This phenomenon is well known as the time-dependent degradation in biological capability or biological degradation, which arises from the absorption of hydrocarbon contaminants in air. In contrast, the contact angles for Ti-AT and Ti-AT-SPT were much lower than that for Ti throughout the aging period. The initial super hydrophilic surface remained almost unchanged up to 30 days of aging in air.

### 3.5 Bioactivity analysis

The number of cells attached to Polished-Ti and Ti-AT-SPT, which were subjected to aging in air for 0, 7, and 30 days, as measured with the hemacytometer. The number of MC3T3E1 cells attached to Ti-AT-SPT was approximately 50% more in amount than that attached to Ti at each aging period, which suggested that the HA coating by solution plasma treatment significantly improved the initial cell attachment ( $p < 0.05$ ). The number of cells attached to the Polished-Ti with 30-days aging in air was approximately 50% of that for the Polished-Ti; without aging. In contrast, the number of attached cells on the Ti-AT-SPT after aging for 30 days remained above 85% of that for the SPT-Ti without aging.

The cells attached to Porous-Ti-AT-SPT after incubation for 4h. The attached cells indicated by arrows were located not only on the outer surface but also on the surface of concavity, which indicated that the induction of bone tissue on the entire porous surface was going to occur successfully and that bone tissue growth could be expected.

## Discussion

### 4. 1 Process of rapid formation of thin and uniform HA film consisting of fine spherical particles on Ti-AT-SPT

With SPT in the calcium phosphate solution, HA spherical particles were precipitated on the Polished Ti surface but the size of the spherical particles was 5 – 20  $\mu\text{m}$  in diameter. The density of the HA crystals was too low to form an HA film. However, it was demonstrated that the thin and uniform HA film consisting of fine spherical HA particles with a diameter of 5  $\mu\text{m}$  was formed on Ti-AT-SPT. This rapid formation of the uniform HA film was attributed to the synergistic effect obtained by combining an alkaline treatment with SPT in a calcium phosphate solution.

The driving force for the formation of HA film from a supersaturated solution was the change in Gibbs free energy,  $\Delta G$ , for transfer from a supersaturated solution to an equilibrium solution with HA crystals:

$$\Delta G = -RT \ln S \quad (1)$$

Where,  $R$  is the gas constant,  $T$  is the absolute temperature, and  $S$  is the degree of supersaturation, which is expressed as:

$$S = IP / K_{sp} \quad (2)$$

Where,  $IP$  is the ionic activity and  $K_{sp}$  is the thermodynamic solubility product. Since the solubility product of HA,  $K_{sp}$ , decreases with increasing temperature, it is apparent that an increase in the degree of supersaturation,  $S$ , together with a decrease in the  $K_{sp}$  value as a result of SPT was responsible for the precipitation of HA crystals on the polished Ti.

SPT for 30 min without alkaline treatment, however, was insufficient for the formation of a dense and uniform HA film. This probably arose from the fact that SPT was not sufficient to lower the activation energy to enhance the formation of HA crystals and a few critical and supercritical HA nuclei were induced by SPT.

With 5 M NaOH treatment, a sodium titanate hydrogel layer formed on the polished titanium. It was also found that the hydrogel layer quickly released sodium ions with the uptake of calcium ions when alkaline treated Ti was soaked in calcium phosphate solution. This ion exchange reaction was proposed to take place very quickly to maintain electrical neutrality of the hydrogel and increased the calcium ion concentration at the hydrogel surface. Increase in IP as a result of a higher calcium ion concentration, also increased the degree of supersaturation with respect to HA, which enhanced the apatite nucleation in a mineralizing solution. In this case, the activation energy required for HA nucleation was also insufficiently lowered by the increased concentration of Ca ions to induce a few nuclei. The HA crystals grew larger with an increase in soaking time in a calcium phosphate solution, and a thick HA film consisting of coarse HA spherical particles was formed after soaking for 1 day at 60 °C.

With the combination of alkaline treatment and SPT, the degree of supersaturation,  $S$ , was markedly increased with both an increase in IP and a decrease in  $K_{sp}$ . The activation energy required for HA nucleation was probably lowered sufficiently to induce a large number of HA nuclei. As a result, a thin and uniform HA film consisting of fine spherical particles could be obtained.

#### 4.2 Chemical and biological properties of HA film formed by SPT

Previous studies revealed that titanium undergoes a time-dependent degradation in biological capability owing to the unavoidable contamination of the titanium surfaces by hydrocarbons in air. Contact angle measurements of the water droplets demonstrated that the titanium surface changed from being hydrophilic to hydrophobic with an increase in aging time. This study demonstrated that the contact angle value for an HA coated titanium disk (Ti-AT-SPT) was significantly lower than that for polished titanium throughout the aging periods. In addition to this, the initial super hydrophilicity of the HA-coated titanium surface by SPT was found to be maintained after 30-days aging in air. Unlike titanium, HA-coated titanium did not show age-related impaired bioactivity, defined as biological aging. The reason for the highly hydrophilic nature of HA-coated titanium can be attributed to the characteristic of HA crystals with the fact that HA is an ionic crystal with an abundance of ionic sites which attract water molecules.

The number of MC3T3E1 cells attached to Ti-AT-SPT was approximately 50% larger than that of Polished-Ti at each aging period, suggesting that the HA coating by solution plasma treatment significantly improved the initial cell attachment. This may be one of the reasons why HA-coated titanium implants possess better cytocompatibility and facilitate rapid bone formation owing to their excellent osteoconductive property.

These results suggest that an osteoconductive porous-surfaced titanium implant can be developed with HA coating by solution plasma treatment in a calcium phosphate solution in a short time.

4.3 HA film coating on titanium by SPT to develop an osteoconductive porous-surfaced dental implant body

HA film coating was achieved when Ti-AT was soaked in calcium phosphate solution at 37 °C for 7 days and at 60 °C for 1 day.

In a 37°C immersion treatment, HA film coating was achieved when polished-Ti and Ti-AT were soaked in calcium phosphate solution for 7days. The approximate size of the precipitated HA spherical particles on Ti-AT was 40–50 µm. In a 60°C immersion treatment, an HA film coating was achieved when Polished-Ti and Ti-AT were soaked in calcium phosphate solution for 24 h. The approximate size of the precipitated HA spherical particles on Polished-Ti was more than 40 µm, and was 20 µm on Ti-AT.

These results suggest that the size of the HA spherical particles precipitated during the immersion treatments in the calcium phosphate solution was too large to coat the titanium implant with a porous surface structure.

With 5 M NaOH treatment and subsequent SPT in the calcium phosphate solution, dense nucleation with critical size nuclei was successfully precipitated on the sample and a thin and dense HA layer consisting of fine spherical crystals was formed on the titanium disk surface after SPT for 30 min .

The solution plasma surface modification for coating HA onto titanium proceeds in a wet process unlike other HA surface methods in a dry process. Therefore, the coating of a HA film consisting of fine crystals on the entire surface of titanium implant bodies with porous surface structures can be achieved. MC3T3E1 cells were observed not only on the outer surface but also on the surface of concavity, indicating that the induction of bone tissue on the entire porous surface was going to occur successfully and that bone tissue ingrowth could be expected. To enhance the growth of mineralized tissue into the

pore spaces and to maintain the vascular system to be interconnected by the pores for continued bone development, the porous structure of a titanium implant provides a unique biological bone anchorage to the titanium implants. Enhancement of bone/implant mechanical bonding can allow dentists to use dental implant bodies with a short length, which can greatly contribute to an increase in the ability of dental implants to be used for a wide variety of treatment options, especially for patients with low bone quality and with advanced maxillary/mandibular residual ridge resorption.

#### 4.4 Features of the SPT for HA film coating

HA film coating on titanium is achieved by combining a simple chemical treatment (5 M NaOH treatment) with SPT in a calcium phosphate solution. One of the striking features of this proposed method was that the rate of HA film formation during SPT was faster than that in most wet processes. For example, Kim et al. reported that a bone-like apatite layer was formed on the 5 M NaOH treated titanium substrate when the substrate had been soaked in simulated body fluid (SBF) for more than 1 day. In contrast, a uniform HA film was observed to form on 5 M NaOH treated titanium by SPT for only 30 min. As stated before, the thermal energy produced during SPT increased the degree of solution supersaturation with respect to HA, and thus greatly increased the growth rate of HA crystals.

The substrate heating method in the liquid with the application of a large current can be applied to electrically conducting materials such as metals and alloys. For SPT, the solution in contact with titanium is heated with the thermal energy generated by plasma, suggesting that this HA coating method can be applied to materials without electrical conductivity such as ceramics. A uniform HA layer can be formed on a polished Y-TZP



surface by SPT in a calcium phosphate solution for 30 min. After pretreatment with 5M NaOH, the formation of a sodium titanate hydrogel was observed on the Ti-AT sample. However, alkaline treatment has no effect on the Y-TZP sample. For HA film formation on Y-TZP, no surface treatment was required prior to SPT, indicating that the activation energy for HA nucleation was lower than that on the polished titanium.

### Conclusion

The present study demonstrated that it is possible to acquire a homogenous precipitation of hydroxyapatite over the entire surface of a smooth and porous titanium disk and zirconia disk by the implementation of a solution plasma surface modification treatment in a mineralizing solution. The solution plasma-treated samples showed significantly better cyto-compatibility than the other specimens.

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