Molecular precursor method for thin carbonate-containing apatite coating on dental implants

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The molecular precursor method is an easy and simple method for coating thin carbonate-containing apatite (CA) films onto titanium surfaces. A molecular precursor solution containing ethanol, calcium-EDTA complex, and phosphate salt was dropped onto a titanium surface and then heated at 600°C for 2 h. An adherent thin CA coating was achieved. Animal implantation experiments showed that CA-coated implants had significantly higher bone-to-implant values than non-coated implants (p<0.05). The molecular precursor method was also used to coat three-dimensional titanium webs (TWs). Thin CA films could be coated inside the center area, as well as the surface of the TW, with excellent bone formation inside the CA-coated TW. Furthermore, the molecular precursor method was used to coat partially stabilized zirconia with CA. Better bone response was observed for CA-coated zirconia. From this, it is concluded that the molecular precursor method is useful for producing thin CA coatings on implant materials.

Keywords: Molecular precursor method, Thin apatite coating, Titanium, Titanium web, Dental implant

INTRODUCTION

Various surface modifications for titanium dental implants such as blasting, or chemical treatments such as an alkaline treatment, have been used to accelerate and improve the bone healing process1-4. Among them, apatite coatings are known to be a useful surface modifier5-8.

The plasma-spray technique is now the most widely used method for the deposition of apatite coatings. However, some shortcomings of plasma-spray apatite coatings, such as degradation and fatigue of the coating, as well as long-term clinical safety and prognosis, have been suggested9-11. To overcome these problems, physical vapor deposition (PVD) techniques such as magnetron sputtering and ion beam dynamic mixing have been introduced to deposit thin calcium phosphate coatings on dental implants12-14. PVD-deposited calcium phosphate coatings are more adherent to the underlying titanium surface and less prone to the formation of cracks than plasma-sprayed coatings. Thin apatite-coated implants prepared by magnetron sputtering are now commercially available in Japan15.

Sato et al.16-18 developed a new coating method for depositing metal oxide films onto ceramics and metal materials. The principle of this new method involves the use of an alcoholic precursor solution of a metal-ethylenediamine-\(N,N',N''\)-tetraacetic acid (EDTA) complex on the substrate, followed by heat treatment of the material at approximately 500 to 700°C. Thin films of TiO2, Co3O4, or SrTiO3 were coated onto a glass substrate using a Ti-EDTA or Co-EDTA complex. The authors called this new technique the molecular precursor method. The semiconductive, transparent Cu2O film with a p-type was fabricated using the molecular precursor method19. Sato et al.20,21 also found that hydroxyapatite can be deposited onto titanium using a precursor solution of a calcium (Ca)-EDTA complex. It was found that the apatite film produced via the molecular precursor method was carbonate-containing apatite (CA). The deposition of a CA film onto a titanium surface is interesting because of its chemical resemblance to bone mineral.

In this review, we introduce the details of a molecular precursor method for coating apatite onto titanium and the bone response. The molecular precursor method is a wet process. We also provide an overview of the applications of the molecular precursor method for coating apatite onto three-dimensional titanium fiber web. The molecular precursor method is herein introduced as a useful apatite coating technique.

THIN CA COATING USING MOLECULAR PRECURSOR METHOD

The molecular precursor method was applied to produce thin CA coatings on titanium disks22-24.

Molecular precursor solution

The procedure for the preparation of a molecular precursor solution for CA coating is shown in Fig. 122-24. First (step 1), a CA-EDTA/amine ethanol solution was prepared. Then, dibutylammonium diphosphate salt ((C4H9)2NH2)2P2O7•2H2O) was obtained from the reaction of 85% mass phosphoric acid and an ethanol solution of dibutylamine (step 2). Finally (step 3), the molecular precursor solution was obtained by adding dibutylammonium diphosphate salt to the Ca-EDTA/amine ethanol solution. The Ca/P ratio was adjusted to 1.67.

Coating procedure

The procedure for coating CA films onto titanium using the molecular precursor method is illustrated in Fig. 2. A molecular precursor solution was added dropwise...
onto a titanium disk surface until the entire area was covered. For example, 20 µL of solution was applied to a titanium disk with a diameter of 12 mm, using a spin coater. The disk was then heated using a tubular furnace under oxygen gas (100 mL/min). Without the introduction of oxygen gas, the coated surface appeared black. The presence of oxygen gas resulted in a relatively white coated surface. Takahashi et al. investigated the effect of heating temperature on the production of a CA coating. They found that heating at 600°C for 2 h is suitable for producing an adherent CA coating on titanium.

Characterization of thin apatite coating
First, the coating surface was observed by electron probe microanalysis (EPMA). Only Ca, P, and Ti were present in the coated film. The coating thickness determined by EPMA was less than 0.5 µm and the Ca/P ratio was 1.56±0.04.

Figure 3 shows the XRD pattern and FT-IR spectrum of the surface of CA-coated titanium. In the XRD pattern, peaks at 2θ=31.8°(211), 33.0°(300), 46.8°(222), and 49.5°(213) were assigned to apatite. The FT-IR spectrum identified the presence of carbonate groups around 1,300–1,500 cm⁻¹. The stretching and bending modes of P-O peaks was observed around 900–1,200 cm⁻¹ and 550–600 cm⁻¹, respectively. Thus, the coating film produced via the molecular precursor method was CA.

The adhesiveness of the CA coated onto titanium was evaluated by tensile bond strength measurement and a scratch test. These tests were performed before and after PBS immersion over 4 weeks. Both tests revealed the excellent adhesiveness of the CA coating on titanium. There was no decrease in adhesiveness or degradation during PBS immersion.

**IN VITRO AND IN VIVO BIOLOGICAL RESPONSES TOWARD THIN CA-COATED TITANIUM**

Biological responses toward the CA-coated titanium were evaluated in vitro, via simulated body fluid (SBF) immersion experiments and in vivo animal implantation experiments. In vitro apatite formation during SBF immersion
As the SBF, Hanks's balanced salt solution (HBSS) without organic species was employed. Greater amounts
of apatite crystal formation were observed on CA-coated titanium compared with non-coated titanium after 1 day immersion in HBSS. The thickness of the precipitated apatite crystals on the CA-coated titanium was almost twice that on the non-coated titanium after 14 days immersion. CA coating increased the apatite formation during HBSS immersion.

**Bone response to CA-coated titanium implant**
Animal implantation experiments were performed. Cylindrical titanium implants were immersed in the molecular precursor solution, then heated at 600°C for 2 h. CA-coated and non-coated cylindrical implants were then implanted in the trabecular bone defects of femoral condyles of rabbits. The bone-implant interface was histologically and histomorphometrically evaluated. Histological appearances of non-coated and CA-coated implants after 12 weeks of implantation are shown in Fig. 4. In the histological appearances, there was no clear difference in bone response between the non-coated and CA-coated implants. The new bone has completely remodeled into mature trabecular bone. On the contrary, the percent of bone-to-implant contact (BIC) ratio of the non-coated implant was 71.3±6.9%, and that of the CA-coated implant was 80.7±5.6%. Statistical analysis revealed that the CA-coated implant showed a significantly higher BIC value than the non-coated implant ($p<0.05$). This indicates that the CA coating produced via the molecular precursor method has excellent bone biocompatibility.

**Regeneration of periodontal ligament (PDL) to CA-coated titanium implant**
Kano et al. evaluated the effect of CA coating and occlusion on the regeneration of a PDL around tooth-shaped titanium implants with root form, in rat animal experiments. CA coating on tooth-shaped implants with root form was performed using the molecular precursor method. They implanted root form implants into the extracted sockets with the remaining PDL of the rat molar model. PDL-like tissue was recognized on the CA-coated tooth-shaped implants. They concluded that occlusal loads to CA-coated implants may induce the regeneration of PDL-like tissue in the peri-implant tissue.

**APPLICATION OF THE MOLECULAR PRECURSOR METHOD TOWARD THREE-DIMENSIONAL TITANIUM WEBS (TWs)**
The molecular precursor method is a wet process. Hence, it may be possible to coat CA films on any shapes of
materials. TW has a unique three-dimensional structure as shown in Fig. 5. The fiber diameter was 50 µm and volumetric porosity was 82%. The average pore size was approximately 200–300 µm. Kuboki et al. proposed that TWs will be a new type of artificial extracellular matrix, and that TWs will be applicable as a non-degradable bone substitute for bone regeneration and a material for cell culture. Kuboki et al. proposed that TWs will be a new type of artificial extracellular matrix, and that TWs will be applicable as a non-degradable bone substitute for bone regeneration and a material for cell culture. Guo et al. placed TW into a rabbit skull defect and concluded that TW promoted the healing of the defected rabbit skull.

Vehof et al. tried to deposit a thin apatite coating on TW using RF magnetron sputtering, which is a representative PVD technique. They reported that the RF magnetron sputter coating did not penetrate completely throughout the TW, and the inside of the TW still consisted of the original titanium. Consequently, the absence of bonding osteogenesis was attributed to the insufficient deposition of the apatite coating. We successfully coated a thin CA film inside the center area as well as the surface of TW using a molecular precursor solution and found that CA-coated TW showed excellent bone formation.

The procedure is also very simple. TW was immersed into the molecular precursor solution for 20 min. Immersed TW samples were placed between filter paper. Paper waste and excess amounts of adsorbed molecular precursor solution were removed. If excess solution is present inside the TW, CA cannot uniformly coat the surface of the TW. Afterwards, the treated TW was heated at 600°C for 2 h using a tubular furnace under oxygen gas. CA was confirmed to be coated inside the TW by EPMA. Ca and P are present not only along the surface area of the TW, but also inside the center area.

Bone formation inside TW was evaluated in animal implantation experiments. Non-coated and CA-coated TWs were implanted into the trabecular bone defects of rabbits or bone defects of rat skulls. Greater amounts of bone formation inside TW were recognized. Figure 6 shows the difference in bone ingrowth between non-coated and CA-coated TW after implantation into the bone defects of rat skulls. The bone formation rate inside TW was 15.1±10.4% for non-coated TW and 59.1±29.2% for CA-coated TW. Amemiya et al. investigated the external bone formation of CA-coated TWs. They placed non-coated and CA-coated TWs under the periosteum of rat calvaria and confirmed greater bone formation and vertical bone formation rates of CA-coated TWs. Hirota et al. tried to use thin CA-coated TWs in the repair of segmental bone defects. They found that CA-coated TW is a bone compatible mandibular reconstruction device in immediately loaded segmental defects. These

Fig. 5  Macroscopic appearance of TW, and SEM images of the fiber structure of TW.

Fig. 6  Histological appearances of bone ingrowth inside TW after implantation into the bone defects of rat skulls.
results confirmed that thin CA-coated TW has better osteoconductivity and will be useful for a non-degradable three-dimensional scaffold.

**APATITE COATING ON PARTIALLY STABILIZED ZIRCONIA**

Partially stabilized zirconia is now being introduced to dental clinics as a new dental material. In implant dentistry, some disadvantages of titanium implants, such as a dark grayish color or metal sensitivity, have been reported. In recent years, yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) has become an attractive material for dental implants as a metal-free system. Thin CA coating was carried out using a molecular precursor method. In the SBF immersion experiment, more apatite deposition was observed on CA-coated Y-TZP at the early stage of immersion in SBF than on the non-coated Y-TZP. Animal experiments revealed that bone formation on CA-coated Y-TZP was histologically similar with that of Y-TZP. However, significantly higher BIC and new bone formation mass on CA-coated Y-TZP was observed after implantation into the trabecular bone defects of femoral condyles of rabbits. It is suggested that CA coating on Y-TZP will improve bone formation around the implant material.

Moreover, the effect of heating conditions, such as heating temperature and heating time, on the formation of CA films onto Y-TZP was evaluated. For example, CA films heated at 800°C for 2 h prior to the immersion in SBF showed greater amounts of apatite precipitation compared with CA films heated at 600°C. The properties of the CA coating on Y-TZP were affected by the heating temperature and heating time.

**CONCLUSION**

The advantage of the molecular precursor method is its simplicity and low cost. There is no need for any specialized or high-cost apparatus. The reagents for the molecular precursor solution are not expensive. Another advantage of the molecular precursor method is that the CA coating can be deposited onto titanium implants of any shape. Moreover, a deposited thin film produced by the molecular precursor method possesses an already crystalline structure, thereby avoiding the use of post-deposition annealing procedures. For example, PVD methods provide amorphous Ca-P coatings, and heat treatment procedures are needed to obtain a crystalline apatitic film.

Another unique approach using the molecular precursor method has been reported by Tokunaga et al. They deposited zirconia films onto titanium using a molecular precursor solution made of a zirconia complex. The molecular precursor method has diverse applications as a coating technology. Hence, the molecular precursor method is useful for coating thin films such as CA onto implant materials.

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