Thin Carbonate-containing Apatite Coating of a Titanium Web using a Molecular Precursor Method under Oxygen Gas Introduction

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SYNOPSIS
Thin carbonate-containing apatite (CA) coatings were deposited onto titanium webs (TW) using a molecular precursor method. The volumetric porosity and fiber diameter of the TW were 87 % and 50 μm, respectively. A molecular precursor solution was obtained by adding dibutylammonium diphosphatate salt to Ca-EDTA/amine ethanol solution by adjusting the Ca/P ratio to 1.67. Two molecular precursor solutions with different calcium ion concentrations, 0.157 mmol/g (0.157-precursor) and 0.470 mmol/g (0.470-precursor), were prepared. Each sintered TW was immersed in the molecular precursor solution and then heated at 600 ºC for 2 hr under oxygen gas introduction. Without oxygen gas, the CA coated TW appeared darker and blacker. Oxygen gas introduction produced a whiter TW appearance after CA coating.

Three-times coating of 0.157-precursor produced a CA coating that covered the surface of the TW as well as its center. By using the 0.470-precursor, a single application of CA covered the inside as well as the surface of the TW.

Increasing the calcium ion concentration of the molecular precursor solution enabled one-time application for effective CA coating not only of the surface of the TW but also its center. In conclusion, one-time application of 0.470-precursor solution during oxygen gas introduction produces a sufficiently CA coated TW.

Key words: molecular precursor method, carbonate-containing apatite coating, titanium web

INTRODUCTION
The molecular precursor method is a new method for coating thin apatite layers onto titanium¹. An alcoholic solution of ethylenediamine-N,N,N,N-tetraacetic acid (EDTA)-calcium (Ca) complex and phosphate compounds is used as a molecular precursor solution. It is reported that heating at 600ºC forms adherent crystalline carbonate-containing apatite (CA) films on titanium substrates using the molecular precursor method²,³. In a very simple procedure, the molecular precursor solution is poured onto the titanium substrate, and then the precursor treated disks are heated for 2
hours. The thickness of the film produced is less than 1 μm. A greater precipitation of calcium phosphate crystals is observed on titanium plates coated with a CA thin film after immersion in simulated body fluid (SBF) compared with uncoated titanium plate surfaces. In vivo animal experiments have demonstrated that titanium cylindrical implants coated with CA film by the molecular precursor method show greater bone-to-implant contact during the healing phase than uncoated titanium.

The molecular precursor method is a wet process. It is considered that it can coat a CA film not only onto flat titanium disks but also onto titanium objects with complex geometry.

Titanium webs (TW), which are produced by sintering intertwined thin titanium fibers, are non-degradable three-dimensional porous scaffold materials that are used as bone graft substitutes. TW have sufficient stiffness and elasticity for this role and are bone compatible and easy to handle during surgery.

Previously, the molecular precursor method has been shown to be able to coat a CA film not only onto the surface of the TW but also inside the TW. CA coated TW showed better bone response compared with uncoated TW after implantation into the trabecular bone of the femoral condyles of rabbits. In previous experiments, the heating procedure in the molecular precursor method was performed under atmospheric conditions. Using this process, it is impossible to completely remove all of the carbon, which is derived from the combustion of organic compounds in the precursor solution during the heating process. Thus, the surface of CA coated TW appear slightly black. Moreover, partial detachment of small amounts of titanium dioxide or coated apatite particles is observed. Such detachment of particles influences the biological properties of the TW, for example the appearances of macrophages or coloration of surrounding tissues, although the details are not clear.

In order to overcome the shortcomings described above, we tried to introduce oxygen gas during the heating process in the present study. The influence of the calcium concentration of the molecular precursor solution was also investigated.

**MATERIALS AND METHODS**

**Preparation of the molecular precursor solution**

The molecular precursor solution for CA coating was prepared by adding dibutylammonium diphosphate salt to a Ca-EDTA/amine ethanol solution. The general procedure for the preparation of the molecular precursor solution is

**Step 1)**

\[
\text{CaH}_{2} (\text{edta}) \cdot 2\text{H}_{2}\text{O} \quad \text{+} \quad (\text{C}_{4}\text{H}_{9})_{2}\text{NH} \quad \rightarrow \quad (\text{C}_{4}\text{H}_{9})_{2}\text{NH} \quad \text{EtOH solution}
\]

**Step 2)**

\[
85 \text{ mass}\% \quad \text{phosphoric acid} \quad \text{+} \quad (\text{C}_{4}\text{H}_{9})_{2}\text{NH} \quad \rightarrow \quad \text{diphosphate salt} \quad \left( (\text{C}_{4}\text{H}_{9})_{2}\text{NH})_{2}\text{PO}_{4} \cdot 2\text{H}_{2}\text{O} \right)
\]

**Step 3)**

\[
\text{Ca}^{2+}=1.67 \quad \text{Co-EDTA/amine ethanol solution} \quad \text{+} \quad \text{diphosphate salt} \quad \left( (\text{C}_{4}\text{H}_{9})_{2}\text{NH})_{2}\text{PO}_{4} \cdot 2\text{H}_{2}\text{O} \right) \quad \rightarrow \quad \text{molecular precursor solution} \quad \text{(Ca}^{2+}=0.157, \ 0.470 \text{ mmol/g)}
\]

*Figure 1. Preparation of the molecular precursor solution*
shown in Fig. 1 and follows the method of previous papers²⁻⁵.

The molecular precursor solution was prepared in 3 steps. During step 1, a Ca-EDTA/amine ethanol solution was prepared from the reaction of CaH₂(edta)·2H₂O and dibutylamine ethanol under reflux conditions. CaH₂(EDTA)·2H₂O was obtained from the reaction of EDTA and Ca(CH₃COOH)₂. A clear Ca-EDTA/amine ethanol solution was obtained. During step 2, dibutylammonium diphosphate salt ((C₄H₉)₂NH₂)₂P₂O₆·2H₂O) was obtained as a white precipitate from the reaction of 85 % mass phosphoric acid and an ethanol solution containing dibutylamine. Finally (step 3), the molecular precursor solution was obtained by adding dibutylammonium diphosphate salt to the Ca-EDTA/amine ethanol solution by adjusting the Ca/P ratio to 1.67.

Two molecular precursor solutions with different calcium ion concentrations, 0.157 mmol/g (0.157-precursor) and 0.470 mmol/g (0.470-precursor), were prepared.

**Titanium web**

Titanium webs (TW, Fig. 2, HI-LEX Corp., Hyougo, Japan) with a volumetric porosity of 87 % and a fiber diameter of 50 μm were used. The diameter of the TW disk was 12 mm and thickness was 2.0 mm. The titanium webs were constructed by intertwining a multiplicity of thin titanium fibers. The fibers are bonded at their points of contact using a sintering process. This results in a very porous interconnected structure.

The fiber structure of TW was confirmed by the observation of field-emission scanning electron microscope (FE-SEM, JSM-6340F, JEOL, Tokyo, Japan) at an accelerating voltage of 5 kV. As shown in Fig.3, the mean pore size of the TW was approximately 200-300 μm.

**Figure 2.** The TW used in the present study

**Figure 3.** FE-SEM views of the TW before CA coating
CA coating
The coating procedure was performed according to the previously described method. Before coating, the TW was cleaned ultrasonically in iso-propanol and then dried at 70°C. Then, the TW was immersed in the molecular precursor solution for 20 min. Subsequently, the treated TW was heated at 600°C for 2 hr using a tubular furnace (EP-KRO-12K, Isuzu Seisakusho, Co., Ltd., Tokyo, Japan) under oxygen gas introduction at a rate of 100 ml/min.

For coating with the 0.157-precursor, the immersion in the molecular precursor solution and heating process were repeated three times (three-time coating). On the contrary, the immersion and heating process were performed only once (one-time coating) for coating with the 0.470-precursor.

After CA coating with the 0.470-precursor, macroscopic observation of the surface appearance of the TW and FE-SEM observation of its fiber structure were performed.

EPMA analysis
The effectiveness of the coating of the CA film inside the TW was evaluated using EPMA (JXA-8200, JEOL, Tokyo, Japan) at an accelerating voltage of 25 kV by detecting the X-ray intensities of Ca-Kα, P-Kα, and Ti-Kα. The specimens were embedded in epoxy resin. After curing the resin, the specimens were cut through their middle using a cutting machine (Finecut, HS-100, Heiwa Tech, Japan) to observe the inside of the TW. These specimens were ground down to 1200 grit, polished using 0.3 μm alumina, and then ultrasonically cleaned with ethanol and distilled water. Afterwards, carbon was coated onto the specimens before the EPMA analysis. The presence of CA coating not only along the surface but also in the center of the TW was confirmed by the elementary mapping of calcium (Ca), phosphorous (P), and titanium (Ti).

RESULTS
The macroscopic appearance of the TW after CA coating with or without oxygen gas introduction is shown in Fig. 4. The TW coated with CA in the absence of oxygen introduction appeared darker and blacker than that coated during oxygen gas introduction. Very small amounts of detached titanium dioxide or apatite particles were observed for the CA coated TW produced without oxygen gas introduction.
introduction, but no detachment was observed for the CA coated TW produced under oxygen gas introduction. Oxygen gas introduction produced a whiter TW appearance after CA coating.

FE-SEM views of the coated TW produced with oxygen gas introduction are shown in Fig. 5. There were no differences in the porous structure or surface appearance of the titanium fibers after CA coating. A pore size of approximately 200-300 μm was almost universally maintained.

Three-times coating of the 0.157-precursor were reported to be effective for CA coating of TW in a previous paper. EPMA analysis was performed to elucidate the effectiveness of CA coating using the 0.470-precursor under oxygen gas introduction.

Figs. 6-9 show the elementary distribution of the TW after CA coating using the 0.157-precursor and the three-times coating method or using the 0.470-precursor and the one-time coating method under oxygen gas introduction. The distributions of P, Ti, and Ca are demonstrated together with an SEM image of TW (SL). Ti mapping shows the structure of the TW.

Three coats of the 0.157-precursor demonstrated the presence of P and Ca not only along on the surface of the TW (Fig. 6) but also in the center of the TW (Fig. 7), which agreed with previous reports.

When using the 0.470-precursor, one-time coating was applied. It found that one-time coating of the 0.470-precursor provided a CA coating along the surface area of TW (Fig. 8) and in the center of the TW (Fig. 9). The present results support the assertion that a thin CA film covering the inside as well as the surface of the TW can be achieved using a 0.470-precursor under oxygen gas introduction and a one-time coating method.

Figure 5. FE-SEM views of the TW after CA coating using the 0.490-precursor under oxygen gas introduction
Figure 6. EPMA analysis of the surface of the CA coated TW after three-times coating with the 0.157-precursor.

Figure 7. EPMA analysis of inside of the CA coated TW after three-times coating with the 0.157-precursor.
Figure 8. EPMA analysis of the surface of the CA coated TW after one-time coating with the 0.470-precursor

Figure 9. EPMA analysis of the inside of the CA coated TW after one-time coating with the 0.470-precursor
DISCUSSION
The present study demonstrated the effective CA coating of TW using a 0.470-precursor under oxygen gas introduction.

TW is now being studied as a scaffold material for bone graft substitution. Pioneering and valuable studies of TW as a scaffold material have been performed by Jansen and his colleagues. The loading of bone marrow stromal cells or growth factor onto TW demonstrated the efficacy of TW as a scaffold material.

Vehof et al. tried to deposit a thin calcium phosphate coating onto TW using an RF magnetron sputtering technique. In their study, they described that RF magnetron sputter coating did not completely penetrate through the fiber structure of the TW and concluded that the insufficient calcium phosphate coating of the inside of the TW influenced the bone response.

The advantage of the molecular precursor method is that CA coating can be deposited onto titanium implants of any shape. Moreover, the deposited thin film produced by the molecular precursor method already possesses a crystalline structure, which avoids 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. Namely, two steps, the deposition of apatitic film and heat treatment crystallization, are needed for PVD methods, while when using molecular precursor methods, a crystalline apatitic film can be deposited in one step.

A biomimetic process using simulated body fluid for apatite coating has also been proposed. Apatite crystals were deposited onto a titanium surface in simulated body fluid. This process also enables the deposition of hydroxyapatite onto any shape of substrate. However, in this process, the titanium has to be treated with NaOH and heated before immersion in simulated body fluid, and it takes several days to form a hydroxyapatite layer on titanium. On the other hand, there is no need for pretreatment of the titanium surface using the molecular precursor method, and apatite deposition only takes a few hours.

For complete combustion of the organic materials in the precursor solution, oxygen gas was introduced during the heating process in the present study. It was revealed that CA coating with oxygen gas introduction produced a whiter TW appearance compared with coating without oxygen gas introduction, and no detachment of titanium dioxide or apatite particles occurred in the CA coated TW. This was due to the improvement of the combustion of organic materials in the precursor solution during heating. Oxygen gas introduction was effective at increasing the quality of the CA coated TW.

Our previous study reported that one-time application of 0.157-precursor was insufficient for CA coating of the inside of the TW and that three-time coating is necessary for complete coating of the TW. In the present study, a 0.470-precursor solution, which had a calcium ion concentration that was three times higher than that of the 0.147-precursor, was used. EPMA analysis suggested that one-time application of CA coating would cover the inside of the TW as well as its surface when using the 0.470-precursor solution. The handling property for CA coating using molecular precursor solution was improved by 0.470-precursor. Increasing the calcium ion concentration of the molecular precursor solution enabled one-time application for effective CA coating not only on the surface of the TW but also inside it.

In conclusion, one-time application
of a 0.470-precursor with oxygen gas introduction produces a sufficiently CA coated TW. CA coated TW can be used as bone substitutes and are also useful for loading cytokines such as BMP or other biological molecules. It is also suggested that thin CA coated TW are well suited to acting as three-dimensional scaffolds for regenerative medicine.

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