Research Article

A Modified Porous Titanium Sheet Prepared by Plasma-Activated Sintering for Biomedical Applications

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1. Introduction

A long segmental bone defects repair is one of the challenging problems in orthopaedic surgery. Although allogenic bone grafts are a current major option [1–3], this technique is associated with problems of significant failure rates, poor mechanical properties, and immunological rejection [2]. Porous materials are of significant importance for bone tissue engineering applications because of the good biological fixation to surrounding tissue through bone tissue [4].

Porous titanium and titanium alloys have been investigated as they provide favourable mechanical properties with an elastic modulus closed to that of natural bone under a load bearing condition [5].

Surface characteristics of porous titanium are important determinants in its scaffold properties since the surface condition of titanium has been reported to play a critical role in bone formation associated with superior osteoblast adhesion and subsequent cell behaviors [6–10].

Recent studies have raised a concern that degradation of biological ability with increase in adsorption of organic impurities on titanium-based biomaterials [6, 7]. This reduces hydrophilicity, adsorption of cell-binding proteins, and subsequent cell functions. Titanium-based biomaterials therefore need to be fabricated as clean surface without particular contamination such as titanium carbide.

There are a number of approaches in fabrication of porous titanium and titanium alloys such as sintering loose titanium powder or fibers, slurry sintering, and also rapid prototyping [3, 4, 11, 12]. Initial surface contamination of sintered porous titanium would be unavoidable event as this is generally processed within the carbon graphite molds under a high thermal pressure [3, 4].

This study aimed to develop a contamination-free porous titanium scaffold by a plasma-activated sintering within an originally developed TiN-coated graphite mold. The surface of porous titanium sheet with or without a coated graphite mold was characterized. The cell adhesion property of porous titanium sheet was also evaluated in this study. The peak of TiC was detected on the titanium sheet processed with the graphite mold without a TiN coating. Since the titanium fiber elements were directly in contact with the carbon graphite mold during processing, surface contamination was unavoidable event in this condition. The TiC peak was not detectable on the titanium sheet processed within the TiN-coated carbon graphite mold. This modified plasma-activated sintering with the TiN-coated graphite mold would be useful to fabricate a contamination-free titanium sheet. The number of adherent cells on the modified titanium sheet was greater than that of the bare titanium plate. Stress fiber formation and the extension of the cells were observed on the titanium sheets. This modified titanium sheet is expected to be a new tissue engineering material in orthopedic bone repair.
2. Materials and Methods

2.1. Specimen Preparation. JIS grade II titanium (KS-50, Kobe Steel, Tokyo, Japan) block was used as starting material. Narrow titanium fibers were made by being turned with diameter approximately 0.4-0.5 mm. The surface of graphite molds were coated with thickness of 1.0 mm TiN by presintering under vacuum with a condition of 1 MPa predisplacement and was processed by plasma-activated sintering under a vacuum at pressure of 20 MPa at 3800 A for 15 sec.

Elemental titanium fibers were filled in the φ 30 mm × 1.0 mm graphite mold with or without a TiN coating. The titanium fibers were subjected to the predisplacement with 1 MPa pressure in the graphite molds. The porous titanium sheet φ 30 mm × 0.5 mm was processed by plasma-activated sintering under a vacuum at pressure of 20 MPa, at 3800 A for 15 sec.

2.2. Surface Characterization

2.2.1. Scanning Electron Microscopy. The surface topographies of the coatings on the specimens were then observed by SEM (S-2360N, Hitachi, Tokyo, Japan).

2.2.2. Thin-Film X-Ray Diffraction (TF-XRD). The crystalline phases of the titanium samples before the tests were detected by TF-XRD (XRD-6100, Shimadzu, Kyoto, Japan) with CuKα radiation. The XRD was operated at 40 kV and 40 mA with a scanning speed of 0.02°/s and a scanning range of 20°-60°.

2.2.3. Number of Cells on Titanium Samples. 10 × 10 × 0.5 mm polished titanium plates and titanium sheets were subjected to cell culture. An osteoblastic cell line, MC3T3-E1, was obtained from the RIKEN Cell Bank (Tsukuba, Japan). Cells were cultured in α minimal essential medium (Gibco) containing 10% fetal bovine serum (Gibco) and 1% antibiotic (penicillin, Gibco) under a 5% CO₂ atmosphere at 37°C. Cells were suspended in the medium at 1 × 10⁵ cells/mL and used for experiments. A 1-mL quantity of floating cells was cultured onto titanium samples at 37°C under 5% CO₂ for 1 d. A cell-counting kit (Dojindo, Kumamoto, Japan) was used for the measurement of cell adhesion. After incubation, each specimen was moved to another well and washed 3 times with PBS (Gibco) to remove nonadherent cells. Adherent cells were mixed with 1 mL of medium and 100 μL of reagent solution. After 1 hr of incubation, the absorbance at 450 nm was measured. The number of adherent cells was calculated from the activity of the original cell suspension.

2.3. Stress Fiber Formation and Cell Morphology. Specimens were placed in 24-well culture plates with 1 mL floating cells each. Subsequently, the specimens were incubated at 37°C in 5% CO₂ for 1 hr. Adherent cells on each specimen after 1 hr of cultivation were dehydrated after being washed with PBS. The cells were fixed with 3.7% formaldehyde in PBS and permeabilized by treatment with 0.1% Triton X-100 (Sigma, Tokyo, Japan) in PBS for 1 min. The cells were then incubated for 3 hrs in a rhodamine-conjugated phalloidin solution. After the cells were washed with water, stress fiber formation and cell morphology were observed with the use of a fluorescence microscope (E-600, Nikon, Tokyo, Japan).

2.4. Statistical Analysis. Results are expressed as mean ± SD (n = 6) within each sample. The normal distribution of each value was confirmed using the Kolmogorov-Smirnov test. The appropriateness of the hypothesis of homogeneous variances was investigated by means of Bartlett’s test. Data were statistically analysed by ANOVA followed by a post hoc Tukey test. A P value of less than .01 was considered significant.

3. Results

3.1. Porous Titanium Sheet. As shown in Figure 1, the porous titanium sheet was structured under the high thermal pressure. The titanium sheet processed with TiN coating was observed to be clean whereas a particular contamination was observed on the titanium sheet processed without TiN coating.
3.2. Surface Characterization. The XRD analysis revealed that the titanium sheet prepared without TiN coated graphite mold showed distinctive TiC peaks while the sheet with coated graphite mold showed TiC peak (Figure 2). The peaks attributable to rutile TiO₂ were detectable on the titanium sheet prepared with TiN coated graphite mold. The peaks attributable to TiO₂ were not detectable on titanium sheet processed without TiN coating.

3.3. Adherent Cells on Titanium Samples. The number of adherent cells on titanium sheet processed with TiN coating was significantly ($P = .002$) higher than that without TiN coating after 1 d (Figure 3). Adherent cells on the titanium sheet with TiN coating had begun to show stress fibers and widely extend, while the stress fiber formation and cell extension on titanium sheet without TiN coating were not distinctive (Figure 4).

4. Discussion

The plasma-activated sintering is a rapid sintering method associated with self-heating phenomena within the powder. This is capable of sintering metal or ceramic powders rapidly to its full density at a relatively lower temperature compared to the conventional furnace sintering methods. The carbon graphite mold has been employed in the plasma-activated sintering due to its electroconductive property and thermostability [3, 4, 11, 13]. The direct heating of graphite mold and the large spark pulse current provide a very high thermal efficiency [14].

The peak of TiC was detected on the titanium sheet processed with graphite mold without TiN coating. Since the titanium fiber elements were directly in contact with the carbon graphite mold during processing; a particular contamination such as TiC is unavoidable event in this condition.

Alternatively, the TiC peak was not detectable on the titanium sheet processed within the TiN-coated carbon graphite mold. This modified plasma-activated sintering with the TiN-coated graphite mold would be useful to fabricate the contamination-free titanium sheet.
Figure 4: Fluorescence microscope images of adherent cells on titanium sheet processed with or without TiN coated graphite mold.

Recent study suggested that adsorption of organic impurities on titanium surface are responsible for reducing the initial cells adhesion, subsequent proliferation and differentiation [6–8]. Amount of carbon absorbed on the titanium sheet seems to be an important part in determining the initial affinity level for osteoblasts and new bone formation.

The present study demonstrated that number of adherent cells on the modified titanium sheet was much greater than that of bare titanium plate. Additionally, stress fiber formation and the extension of the cells were observed on the titanium sheets. The initial adhesion of cells induces stress fiber formation, phosphorylation of focal adhesion kinase, and activation of other intracellular signal transduction molecules thereby affecting cell proliferation, differentiation and new bone formation [15]. Thus, the contamination-free surface of modified titanium would be useful for new bone generation at a segmental bone defect in comparison with the unmodified sintered porous titanium.

In conclusion, the TiN-coated carbon graphite mold is a new method for processing a contamination-free porous titanium sheet. This modified titanium sheet is expected to be a new tissue engineering material in orthopedic bone repair.

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