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Formation and Properties of Transparent Titanium Thin Films by DC Magnetron Sputtering for Observing Interactions between Titanium and Cells

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Transparent titanium thin films with flat surfaces were uniformly formed on inner surfaces of tissue culture dishes with the inside diameter of 50 mm by DC magnetron sputtering method with DC power of 300 W for 30 s in 2.2×10^{-1} Pa Ar gas using commercially pure titanium target after 300 s pre-sputtering. After forming the films on the dishes, the dishes were not broken and did not decompose. The dishes with thin titanium films had light black color and black lines printed on a white paper behind the dishes were clearly seen by eyes. The dishes with thin titanium films have around 10% transmittance in the wavelength between 400 nm and 800 nm. All the color can be observed in the range of visible light. When cells are dyed, one can observe the color of cells. After culturing mouse osteoblast (MC3T3-E1) cells for 24 h on the dish, the films on the dishes were not broken and did not decompose. Living cells on titanium film were observed from the back of the dish by the inverted optical microscope. This method is useful for observations of the interactions between cells and titanium oxide on titanium metals.
Key words: titanium, thin film, DC magnetron sputtering method, mouse osteoblast cell, tissue culture dish

1. INTRODUCTION

Commercially pure (cp) titanium and titanium alloy are useful for biological application such as dental, surgical and orthopedic implants [1-4], because the titanium materials have excellent mechanical properties and biocompatibility [5-7]. In the case of using general titanium implants instead of living bone, titanium oxide layer on the titanium is fixed within a bone bed through direct attachment to living bone (osseointegration) [8]. In order to understand biological functions of the titanium materials, interactions between titanium oxide and tissue are important [9]. When we try to observe the interaction from upper side, cells are in our way, as shown in Fig. 1. When we try to observe the interaction from the side, we can see very small region. In the case of transparent materials such as glass, it is possible to observe interface between the material and cells from the back of the material by an optical microscope. However, it is difficult to observe interactions between cells and titanium oxide on the titanium metal materials, because the titanium metal materials are not transparent. Therefore, it is generally difficult to observe the interaction between cells and titanium materials in detail.

In this study, we report formation of transparent titanium thin films for observing the interactions between titanium and cells. In order to form the surfaces of cp titanium implants transparently, thin titanium films were formed on inside surfaces of tissue culture dishes by DC magnetron sputtering method. In this case, for simulating the surface of titanium implants, it is preferable to reproduce thin titanium below titanium oxide that is naturally made in the air. Then, mouse osteoblast (MC3T3-E1) cells were normally cultured on the dishes. After 24 h, the samples were observed from the direction of the cells' bottom surfaces adhered the titanium oxide on the thin titanium film by an inverted optical microscope.

2.EXPERIMENTAL

Tissue culture plastic dishes (Becton Dickinson, FALCON) with the inside diameter of 50 mm were used for substrates. Cp titanium thin films were deposited on inner surfaces of the dishes by DC magnetron sputtering

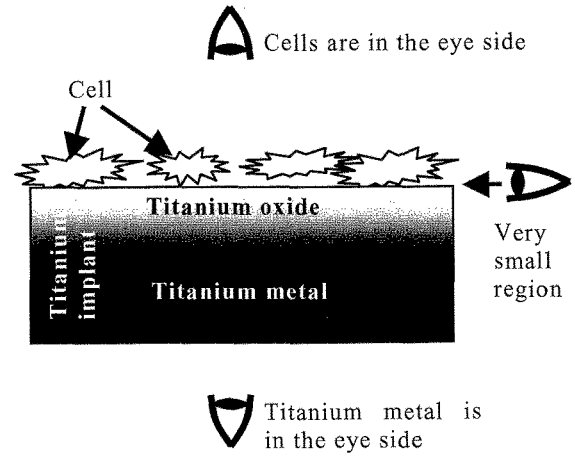

Fig. 1. Observation direction for studying interaction between cell and titanium metal implant.

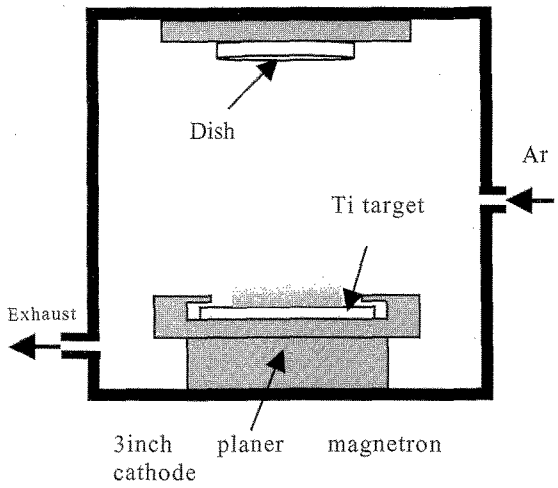

Fig. 2. Schematic illustration of the DC magnetron sputtering for the deposition of cp titanium film onto tissue culture dish.

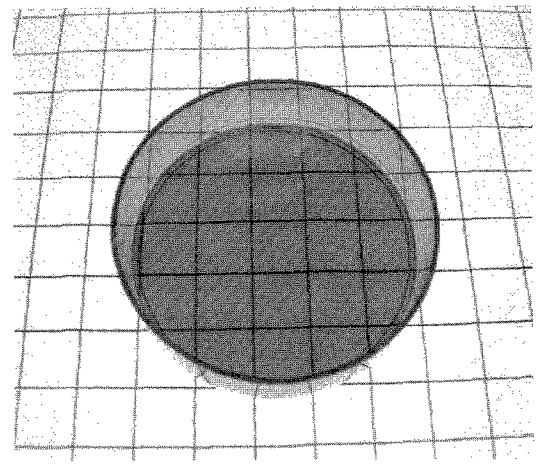

Fig. 3. Photograph of typical tissue culture dish with thin titanium film on a white paper printed lines at intervals of 10 mm.

apparatus (ANELVA corporation, L332S-FHS), with DC power of 300 W for 30 s in 2.2×10^{-1} Pa Ar gas using cp titanium target (99.9%, Kojundo Chemical Lab. Co.,Ltd) after 300 s pre-sputtering to clean the target surface and the vacuum chamber inside for eliminating contaminations. Cp titanium films with about 30 nm thick are formed under the above depositing conditions [10,11]. The DC magnetron sputtering machine has 3inch planer magnetron cathode for decrease of thermal damage to sample, as shown in Fig. 2. Also, the substrate dishes were placed in a downward direction on an upper sample holder at 90 mm above the cp titanium target for keeping clean surface of the dishes. The dishes were observed by eyes and transmittance of the samples were measured by a spectrophotometer (V570, Jasco Inc.). Surfaces of the samples are measured by a scanning electron microscope (SEM, S3500N, HITACHI, Ltd.) and an electron probe micro-analyser (EPMA, JXA-8900L, JEOL Ltd.). Analyzing crystals for EPMA qualitative analysis were thallium acid phthalate (TAP), Pentaerythritol (J) (PETJ), Pentaerythritol (H) (PETH) and layered dispersion element 2 (LDE2).

Roughly 4×10^4 MC3T3-E1 cells cultured on the dish with alpha-minimal essential medium (α-MEM) supplemented with 100 units/ml penicillin and 100 μg/ml streptomycin in 5% CO_2. After 24 h, the dishes with the cells were observed by an inverted optical microscope (IX71, OLYMPUS Co.).

3.RESULT AND DISCUSSION

A photograph of typical tissue culture dish with thin titanium film on a white paper printed lines at intervals of 10 mm is shown in Fig. 3. Thin titanium film was uniformly formed on the surfaces of the tissue culture dish. The dish with titanium thin film had light black color, and black lines printed at intervals of 10 mm were clearly seen by eyes. Therefore, the dish had an enough transparency to observed things behind the dish. The film did not have complete transparent because all of titanium in the film was not oxidized and titanium metal remains under the titanium oxide layer. Therefore, the surface layer of the film have the same layer surface

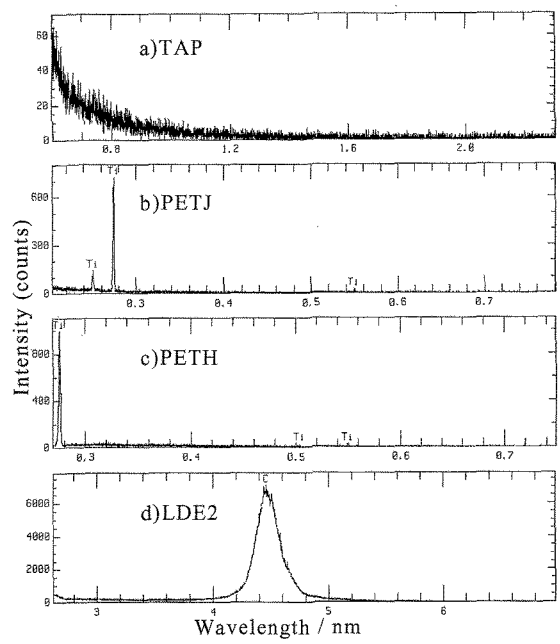

Fig. 4. EPMA qualitative analysis results of the surface of typical tissue culture dish with thin titanium film.

structure as that of general titanium implants.

EPMA qualitative analysis results of the surface of typical tissue culture dish with thin titanium film is shown in Fig. 4. Ti peaks were observed excepting broad C peak from the tissue culture dish. This result indicated that there is surely titanium thin film on the tissue culture dish.

A transmittance of typical tissue culture dish with thin titanium film is shown in Fig. 5. The dishes with thin titanium films have around 10% transmittance in the wavelength between 400 nm and 800 nm. This result indicates that all the color can be observed in the range of visible light. When cells are dyed, one can observe the color of cells. This optical property of the dish with cp titanium films is useful for experiments on tissue

culture.

A SEM photograph of the surface of typical tissue culture dish with thin titanium film is shown in Fig. 6. A white line in the upper part of the SEM photograph was a dust for focusing. The film was uniformly deposited on the dishes and the surface of the film was flat. Though the plastic dish is easy to decompose by thermal damage, the dish was not broken and did not decompose.

Typical optical micrograph of 24 h cultured MC3T3-E1 cells observed from the back of the tissue culture dish with thin titanium film by an inverted optical microscope is shown in Fig. 7. White lines might be a fingerprint or scales on the reverse side of the dish. Living cells on titanium film were observed from the back of the dish. The films on the dishes were not broken and did not decompose after culturing for 24 h. Then, The cells were normally cultured on the film. As result, we could observe the dish with living cells from the direction of the cells' bottom surfaces on titanium by the inverted optical microscope.

4.CONCLUSIONS

Transparent titanium thin films with flat surfaces were uniformly formed on inner surfaces of tissue culture dishes by DC magnetron sputtering method using cp titanium target. Though the plastic dishes are easy to decompose by thermal damage, the dishes were not broken and did not decompose. The dishes with titanium thin films had light black color, and lines printed at intervals of 10 mm behind the dishes were clearly seen by eyes. The dishes with thin titanium films have around 10% transmittance in the wavelength between 400 nm and 800 nm. This result indicates that all the color can be observed in the range of visible light. When cells are dyed, one can observe the color of cells. This optical property of the samples is useful for experiments on tissue culture.

Living cells on titanium film were observed from the back of the dish. The films on the dishes were not broken and did not decompose after culturing for 24 h. Then, The cells were normally cultured on the films. As result, we could observe the dish with living cells from the direction of the cells' bottom surfaces on titanium by the inverted optical microscope. Therefore, this method is useful for observations of the interactions between cells and titanium oxide on titanium metals.

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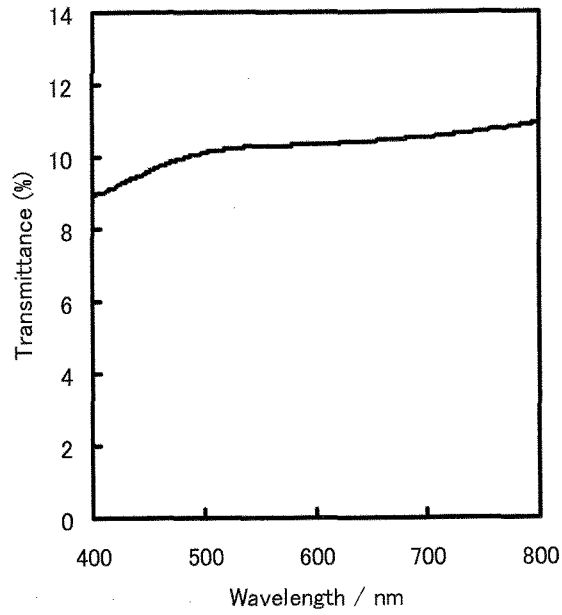

Fig. 5. Transmittance of typical tissue culture dish with thin titanium film.

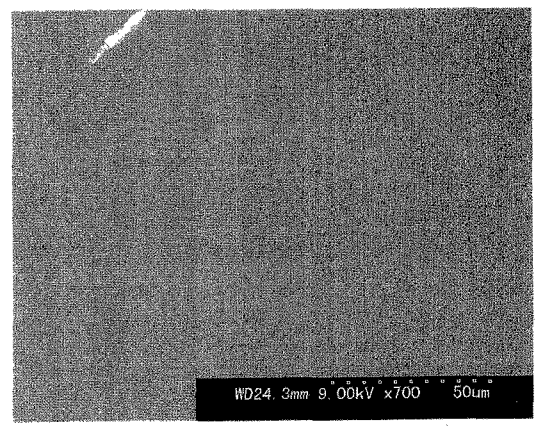

Fig. 6. SEM photograph of the surface of typical tissue culture dish with thin titanium film.

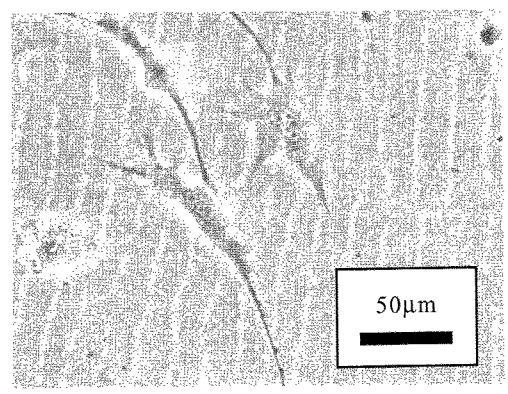

Fig. 7. Typical optical micrograph of 24 h cultured MC3T3-E1 cells observed from the back of the tissue culture dish with thin titanium film.

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