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Surface characteristics and cell-adhesion performance of titanium treated with direct-current gas plasma comprising nitrogen and oxygen

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ABSTRACT

In this study, we attempted to form titanium oxynitride (TiO_xN_y) layers on titanium (Ti) surfaces using direct-current (DC) plasmas generated from gas mixture comprising hydrogen, nitrogen, and oxygen. Additionally, the effect of gas mixture ratio on the surface characteristics and cell-adhesion performance was investigated. Scanning probe microscopy (SPM) images showed that the plasma-treated surfaces were slightly rougher than untreated Ti surfaces, owing to the formation of new layers. Chemical state analysis using X-ray photoelectron spectroscopy (XPS) revealed that the layers were comprised TiO_xN_y, titanium nitride (TiN), and titanium dioxide (TiO_2); the concentrations of TiO_xN_y and TiN decreased and that of TiO_2 increased with an increase in the amount of oxygen in the gas. An increase in the amount of oxygen gas did not affect the layer thickness, which was approximately 25 nm. Furthermore, no differences in cell morphology and cell-adhesion performance were found between the specimens treated with various plasma gases. This is probably because the treatment insufficiently improved the hydrophilicity. Layers composed of TiO_xN_y, TiN, and TiO_2 were formed using the DC plasma treatment; however, the layers did not improve the cell-adhesion at an initial stage after the seeding.

1. Introduction

Titanium (Ti) and its alloys are widely used as orthopedic and dental devices because of their low cytotoxicities and excellent mechanical properties [1–4]. However, Ti-based materials do not rapidly bond to bone after they have been implanted in the human body. Recently, titanium oxynitride (TiO_xN_y) coatings were suggested to enhance the biocompatibility of Ti-based materials, thereby overcoming the aforementioned weakness. Durual et al. fabricated a TiO_xN_y layer on a Ti substrate using a sputtering deposition technique, and they found that cell proliferation and cell differentiation on the TiO_xN_y-coated surface significantly improved in comparison to those on an untreated surface [5,6]. In addition, they reported that the formation of new bone around the TiO_xN_y-coated Ti implant was facilitated in vivo [7]. Tsyganov et al. also evaluated the cellular response on TiO_xN_y layer formed by the ion implantation technique, and they found that cell proliferation accelerated in comparison to that on a titanium dioxide (TiO_2) layer [8]. Furthermore, Banakh et al. prepared TiO_xN_y layers of various chemical compositions using a sputtering deposition technique, and they found that osteoblast cells proliferated and extended on all of the coated surfaces, regardless of the chemical composition of the layer [9]. However, coating substrates with complex shapes are far from easy with these techniques; additionally, the instruments are expensive because they require high vacuums.

Plasma treatment can be performed under a medium vacuum; therefore, the price of a plasma-treatment instrument is comparatively low. Furthermore, this technique can easily treat the entire surface having complex shapes. Yoshinari et al. reported that the hydrophilicity of Ti substrates improved by exposing them to oxygen gas plasmas, thereby enhancing the cell adhesion to the surfaces [10]. On the other hand, Souza et al. reported that treatment of Ti surfaces with gas plasmas comprising hydrogen and nitrogen resulted in the formation of titanium nitride (TiN) layers [11]. Based on these studies, we hypothesized that treatment of a Ti substrate with a plasma generated from a mixture gas comprising hydrogen, nitrogen and oxygen forms a hydrophilic TiO_xN_y layer, and that this layer enhances both the cell adhesion and cell proliferation.

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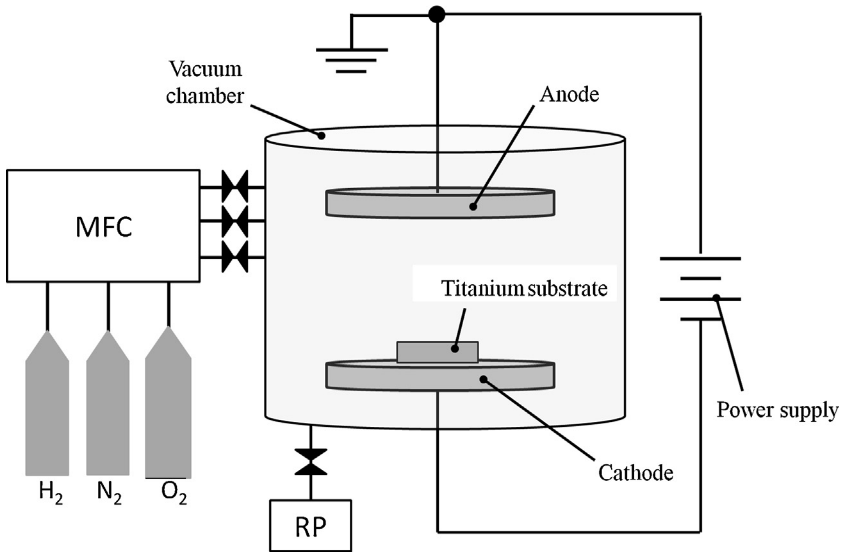

Fig. 1. Schematic illustration of the direct-current plasma-treatment instrument.

Table 1
Gas mixture ratios used for the plasma treatment and names of the specimen fabricated in this study.

Specimen	Hydrogen flow (%)	Nitrogen flow (%)	Oxygen flow (%)
$0\%O_2$	90.0	10.0	0
$0.2\%O_2$	90.0	9.8	0.2
$0.5\%O_2$	90.0	9.5	0.5
$1.0\%O_2$	90.0	9.0	1.0
$5.0\%O_2$	90.0	5.0	5.0

The ultimate goal of our research is to develop a novel surface-modification technique, using a gas plasma comprising hydrogen, nitrogen, and oxygen, for enhancing the cell-adhesion, proliferation and differentiation performances simultaneously. For this purpose, in this study, we fabricated TiO_xN_y layers on Ti substrates using gas plasmas comprising hydrogen, nitrogen, and oxygen in various mixture ratios, and we investigated surface characteristics such as surface morphology, roughness, and chemical states in detail. Furthermore, we evaluated the cellular response on the treated surfaces. Together with these results, we discuss the effect of the plasma treatment on the surface characteristics and the cellular response.

2. Experimental procedures

Ti substrates with sizes of $10\,mm \times 10\,mm \times 1\,mm$ and $\phi 15\,mm \times 1\,mm$, polished using a colloidal silica suspension, were used as substrates. The former size was used for surface analyses and the latter size was used for in vitro tests. Direct-current (DC) glow-discharge plasma treatment was conducted using an instrument built in our laboratory (Fig. 1). Cathode and anode electrodes made of titanium and stainless steel, respectively, were placed parallel to each other at a distance of 20 mm. Diameters of the cathode and the anode electrodes were $\phi 61$ and $\phi 101$ mm, respectively. The Ti substrates were placed at the center of the cathode, after which the chamber was evacuated up to 6.0×10^{-2} Pa. Hydrogen, nitrogen, and oxygen gases were introduced into the chamber through mass-flow controllers. Gas mixture ratio used in this study and the name of the fabricated specimens are summarized in Table 1. The plasma was discharged at a constant pressure of 70 Pa and a power of 50 W, and the substrates were exposed to the plasma for 10 min

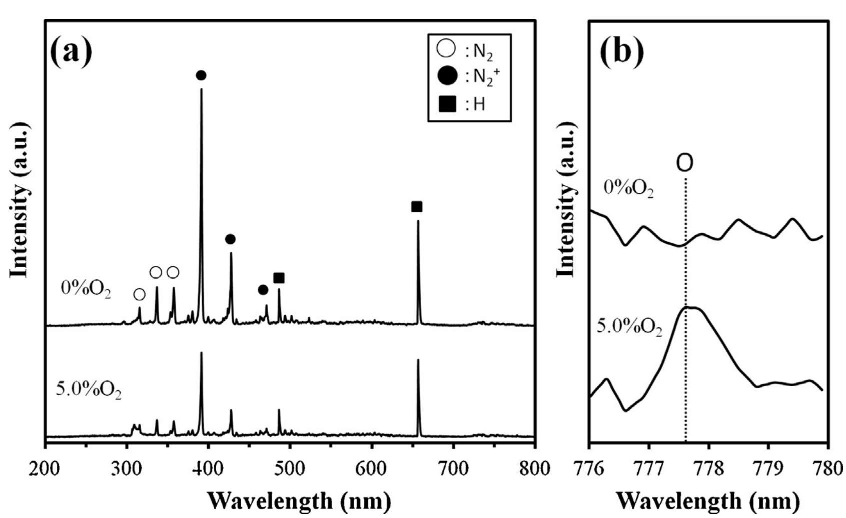

Fig. 2. Optical emission spectra of plasmas using mixture gas corresponding to $0\%O_2$ and $5.0\%O_2$ specimens. (a) Full spectra and (b) spectra magnified in the range of 776–780 nm.