Micro-Arc Oxidation Treatment for Improvement of Antibacterial Property of Titanium

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Statement of Purpose: Titanium (Ti) is widely used in both orthopedic and dental fields due to their good mechanical properties, high corrosion resistance, and biocompatibility. Micro-arc oxidation (MAO) is a useful surface treatment based on electrochemical reactions under high voltage in a specific electrolyte. MAO treatment can easily alter the surface properties of metals and is also effective to improve hard tissue compatibility of Ti. Our previous study revealed that certain amounts of additional elements presented in the electrolyte are incorporated in the porous oxide layer during the MAO process. Therefore, in this study, MAO treatment was performed on Ti in silver (Ag)-containing solution. The incorporation of Ag in the porous oxide layer may lead the slight release of Ag ions from the oxide layer and finally antibacterial property may be achieved after implantation in a living body. The surface oxide layer of MAO-treated Ti was characterized by detail surface analyses. The behavior of the Ag-ion release from the oxide layer was evaluated by immersing in a simulated body fluid. ICP-AES was performed to determine the concentration of Ag ions in the fluid. In addition, the antibacterial property of the samples was evaluated.

Methods: A commercially pure Ti rod was sliced to make disk-shaped specimens with 8 mm in diameter and 1.5 mm in thickness. The surfaces of the disks were grinded with up to #800 grid SiC abrasive papers followed by ultrasonic cleaning in acetone and ethanol. The specimen was fixed in a polytetrafluoroethylene holder that allows exposing to an electrolyte only the specimen surface (7.0 mm in diameter). A Type-304 stainless steel plate was used as a counter electrode. The electrolyte for MAO treatment was 0.1-mol L^{-1} calcium glycerophosphate, 0.15-mol L^{-1} magnesium acetate, and 0-10 mmol L^{-1} silver nitrate. After pouring the electrolyte into the electrochemical cell, positive voltage was applied for 8-10 min. The surface morphologies and compositions of the specimens were analyzed using a scanning electron microscope with an energy-dispersive X-ray spectroscope (SEM/EDS). X-ray diffraction (XRD) was also performed with a diffractometer to identify the crystal structure of the oxide layer. 0.9mass% NaCl solution was used as a simulated body fluid to evaluate the Ag ion release from the MAO-treated specimen. The solution was replaced every 7 d to simulate the refreshment of the body fluid inside a living body. ICP-AES was used to measure the concentration of the Ag ions in the solution.
Antibacterial properties of the samples were evaluated by *Escherichia coli* culturing method (ISO 22196:2007). Colonies of the bacteria was counted after 24 h incubation on the treated or untreated specimens.

Results: The specimen after MAO treatment in the Ag-containing electrolyte showed porous oxide layer on its surface (Fig.1). The morphology of the layer was not changed by the presence of Ag ions in the electrolyte. However, the voltage was not fully raised when the Ag concentration of the electrolyte exceeded 5 mmol L^{-1} so that it was unable to form the oxide layer. The EDS results showed that Ag was incorporated in the oxide layer. Ag-ion release was confirmed by the dissolve test in simulated body fluid (0.9mass%NaCl aqueous solution). Although the release rate of Ag ion was relatively fast at the initial stage of the immersion, it was gradually stabilized to be a certain value about 5 mgm^{-2} per week and it was kept for at least 4 months. Thus, we confirmed the long-time Ag ion release from the incorporated Ag in the oxide layer by this technique. Figure 2 shows the result of the antibacterial evaluation test. The calculated initial count of the bacteria was $1.3x10^{-5}$ CFU/mL. Therefore, *Escherichia coli* could proliferate on both untreated and MAO-treated Ti without Ag. On the other hand, number of the viable bacteria apparently decreased when it was incubated on the MAO-treated Ti with Ag. Thus, we confirmed that antibacterial property can be added on Ti by this technique.

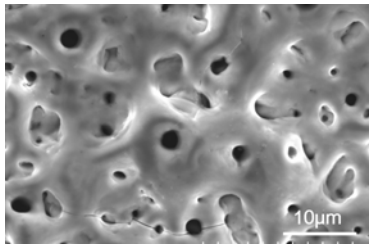

Figure 1. SEM image of the Ag-containing porous oxide layer formed on Ti by MAO treatment

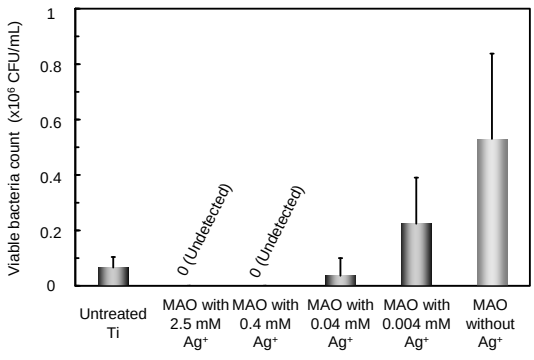

Figure 2. Results of antibacterial evaluation test of MAO treated and untreated Ti using *Escherichia coli.*

Conclusions: We succeed to make the Ag-containing porous oxide layer on Ti. Ag ions were released from the sample when it was immersed in a simulated body fluid and it showed antibacterial property. Thus, this technique is useful to develop novel biomaterials having both hard-tissue compatibility and antibacterial propery.