A novel plasma surface treatment to suppress bacterial adhesion on titanium alloy

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Introduction

Metallic materials for orthopaedic implantation are usually made of titanium alloys due to high mechanical strength, corrosion resistance, low modulus and superior fatigue properties as well as good biocompatibility [1]. However, post-operative implant related bacterial infection is uncommonly seen. This may eventually lead to implant removal if antibiotic therapy fails. Bacterial adhesion on implant surface is an important virulent factor in local implant-related infection. To avoid bacterial infection, the primary line to defense is to inhibit the initial adhesion of bacteria on the implant surface. If maintained, suppression of subsequent bacterial proliferation and biofilm formation will be possible. Surface treatments such as antibiotic coating and silver-cupper ion doping have been proposed [2, 3]. However, precise control of antibiotics release is challenging and its antimicrobial effect is not long-lasting. Release of silver and copper ions may take collateral damage to surrounding cells. Recently, titanium dental implants that have been treated with nitrogen physical vapor deposition (PVD) demonstrate improved antibacterial ability [4]. Therefore, this study aims at suppressing bacterial adhesion on orthopaedic implant surface using plasma surface technology. Plasma immersion ion implantation (PIII) is a viable surface technique to modify the material surface including surface topography and chemistry.

Methodology

The discs made of medical grade Ti-6Al-4V alloys measured in 5 mm in diameter and 1mm in thickness were prepared for PIII treatment. Oxygen plasma surface treatment was carried out in the plasma immersion ion implanter. The parameters of oxygen PIII are summarized in Table 1.

	Working pressure (Torr)	Voltage (kV)	Repetition frequency (Hz)	RF power (W)
S1	5×10^4	40	50	1000
S2	5×10^4	40	100	1000

Table 1 Parameters of oxygen PIII implantation

The elemental depth profiles were determined by X-ray photoelectron spectroscopy (XPS,Physical electronics PHI 5802, Minnesota, USA) with the use of aluminum X-ray source with 350W, and the surface topography and roughness were determined by atomic force microscopy (Auto Probe CP, Park Scientific Instruments). The bacterial adhesion was assessed by counting colony forming unit (CFU) using *S.aureus* culture. The number of cells was determined by surface plating on Brain Heart Infusion agar after 24hrs incubation. LIVE/ DEAD BacLight Viability Kit staining (Invitrogen, Molecular Probe) was applied to evaluate the adhered bacteria morphology.

Results and discussion

The XPS depth profiles confirm that oxygen was implanted into the substrates. The thickness of the oxide layer ranged from 259 nm to 288 nm for implantation frequency of 50Hz to 100Hz, respectively. Surface topography and roughness were significantly changed after treatment (Figure 1). The root-mean-square of the control was 40Å, whereas the O-PIII 50 Hz and 100Hz samples were 22Å and 24Å, respectively. Smooth surface was obtained after oxygen PIII. The number of attached bacteria on the control was doubled as compared with the PIII samples (Figure 2). However, the variation between 50Hz and 100Hz samples was insignificant. Figure 3 visualized the attached bacteria on the O-PIII and control samples under the fluorescent microscope. Green spot represents living bacteria.

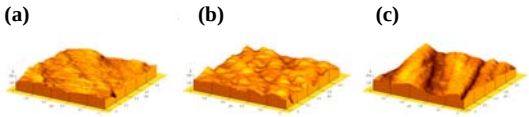

Fig.1 Surface morphology of (a) OPIII 50Hz, (b) OPIII 100Hz & (c) the control

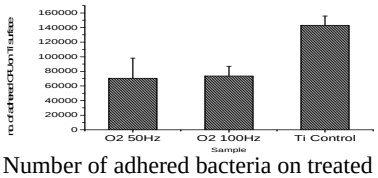

Fig.2 Number of adhered bacteria on treated and untreated Ti surface

Fig.3 Fluorescent microscopic view of (a) O-PIII 50Hz (b) O-PIII 100 Hz and (c) control after 1 hour culture

Oxygen plasma treatment produces a compact oxide layer on the surface and higher implantation frequency results in a thicker modified layer and smooth surface. Previous literatures reported that rough surface directly contributes to bacterial adhesion [5-6]. Oxygen PIII can help smoother titanium surface, thereby resulting in the reduction of bacterial adhesion. However, it also depends on other factors such as surface chemistry and the biological environment. Oxygen plasma treatment can establish TiO_2 on the Ti surface. Previous study recognized titanium oxide can reduce bacterial adhesion as well [7]. In addition, plasma treated antibacterial surface not depends on the release of antibiotics and metallic ions to kill bacteria. Therefore, the antibacterial mechanism is long-lasting.

References

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