Introduction: Metallic materials continue to play an essential role as biomaterials in repairing or replacement of bone tissues that have become diseased or damaged[1]. Various metals such as titanium alloy are widely applied to surgical implantation because of its high mechanical strength, corrosion resistance and superior fatigue properties as well as bio-compatibility[2]. However, debridement of implant due to bacterial infection is sometime seen in bone fracture fixation and deformity correction. Bacterial adhesion on the implant surface is considered to be an important event in the pathogenesis of bacterial infection. To avoid bacterial infection, surface modifications have been conducted on biomaterials so as to achieve antibacterial activity. For instance, silver and copper plasma immersion ion implantation (PIII) surface treatments have been used in the metallic and polymeric substrates to inhibit the bacteria attachment[3-4]. Antonio et al.[5] reported that the titanium dental implant with nitrogen physical vapor deposition (PVD) surface treatment demonstrates antibacterial ability in dental application. Plasma surface modification is actually a viable technique to modify the surface of medical and dental implants. It can be used to tailor make the surface topography, surface chemistry, and modified layer thickness of material. In this study, rather than the dental application, we aim to explore the feasibility of antibacterial ability of nitrogen PIII treated titanium alloy in orthopaedics.

Materials and Methods: Titanium alloys (Ti-6Al-4V) were used, since this material has been applied in orthopaedic implantation. The Ti discs measured in 5mm in diameter and 1mm in thickness were prepared for PIII. All the specimens were mechanically polished with SiC papers before implantation, and then followed by ultrasonic cleaning in acetone and deionized water and dried in air. Nitrogen PIII surface treatment with working pressure (5X10⁻⁴ torr), 1000W RF power and different voltage and frequency applied (20, 30 & 40kV, 60, 100 & 200Hz) were carried out in the plasma immersion ion implanter at City University of Hong Kong.

The elemental depth of the untreated and plasma modified samples were determined by X-ray photoelectron spectroscopy (XPS, Physical electronics PHI 5802, Minnesota, USA) with the use of aluminum X-ray source with 350W. The take-off angle was 45° and the base vacuum was 2x10⁻⁸Pa. The scanning area was about 50mm². The depth profiles were obtained by argon ion sputtering and the estimated sputtering rate was about 19.88nm/min.

The surface topography and roughness of the control and N-PIII Ti surfaces were determined by atomic force microscopy (AFM, Auto Probe CP, Park Scientific Instruments) using contact mode under room temperature and scanning area was 5μm².

The adhesion of bacteria on the control and treated surfaces was assessed by counting colony forming units (CFU) with the use of S. aureus. Medium with 20μl (about 1X10⁸/ml CFU) of overnight S. aureus culture were added onto each of sample surfaces. They were then incubated in 37°C with supplement of 5% CO2 for 1 hour. Afterwards, sample surfaces were rinsed with 1ml PBS for three times in order to remove unattached bacteria. The attached bacteria were then detached by adding 1ml 0.01M PBS containing 0.01% Tween 80 with the use of sonication for 1 minute. The number of detached S. aureus was determined by surface plating on Brain Heart Infusion agar and then incubated at 37°C for 24hrs.

Results: The elemental depth profiles suggest that various thicknesses of nitrogen-rich layers can be achieved by different parameters of nitrogen modified treatments. The thickness of nitrogen-rich layers ranges from 19.88nm to 99.4nm by using 20, 30 and 40kV (data not shown). A higher concentration of nitrogen can be obtained by higher voltage and frequency. This phenomenon also represents the nitrogen is successfully implanted into the Ti substrate.

The root-mean-square (RMS) values represent the surface roughness of the samples. The RMS of the control is 193Å and the range of the RMS of N-PIII samples is 228Å to 293Å. The roughest surface is resulted by higher voltage and frequency. In comparison of the RMS value, the surface of control is smoother than the N-PIII modified one.

The numbers of attached bacteria of various N-PIII samples are about 3 times higher than the control by the count of CFU.

Fig.1 displays the bacteria morphologies of the control (a) and N-PIII sample with 20kV 60Hz (b) observed under fluorescent microscopy. Red color represents dead bacteria and green color indicates live one. N-PIII(b) surface demonstrates relatively more attached live bacteria than control. Higher density of attached bacteria in N-PIII sample is also observed.

Discussion: This study indicates that nitrogen plasma immersion ion implantation titanium alloy does not have an ability to reduce S. aureus adhesion. It may suggest that PIII treatment obviously alters the surface roughness and morphology. It also seems that rough surface does favor S. aureus adhesion. In comparison with the nitrogen plasma treated titanium dental implants with the use of physical vapor deposition[5]; N-PIII treated implant is not able to resist bacterial adhesion. Our findings contradict with the study of Antonio and his associates. Perhaps the nitrogen plasma treated titanium surface can resist the mixture of oral bacteria and the salivary proteins attachment, but not applicable to S. aureus. Nitrogen plasma modified titanium surface is able to resist bacterial adhesion in dental application. However, this modified surface is not able to resist S. aureus adhesion in which this bacteria is commonly found in orthopaedic infection. Regardless of better surface coating with long lasting modified surface and good biocompatibility of cell[6], N-PIII modified titanium surface is not able to inhibit S. aureus adhesion.

References:

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