A NEW NITROGEN PLASMA IMPLANTED TITANIUM SURFACE TO INHIBIT ORTHOPAEDIC RELATED INFECTION

Leung KY[1], Kao RYT[2], Chu PK[3], Luk KDK[1], Cheung KMC[1], Yeung KWK[1,3#]

[1] Division of Spine Surgery, Department of Orthopaedics and Traumatology, Queen Mary Hospital, The University of Hong Kong, HKSAR, China

[2] Department of Microbiology, Queen Mary Hospital, The University of Hong Kong, HKSAR, China

[3] Department of Physics and Materials Science, City University of Hong Kong, HKSAR, China

Introduction: Orthopaedic metallic implants are usually made of medical grade pure titanium and titanium alloys because of their high mechanical strength, corrosion resistance and superior fatigue properties as well as biocompatibility [1]. Bacterial infection is sometime seen in trauma fixation surgery and joint reconstruction due to bacterial growth on the implant surface. To minimize bacterial growth and biofilm formation, suppression of bacterial adhesion is a key to success. Surface treatments such as antibiotic coating and silver ion doping have been adopted due to the ease of fabrication. However, control of antibiotics release is challenging and the release does not last too long. Therefore, our group aims to another alternative. Recently, combinations of silver and copper ion surface coatings have been applied to the metallic and polymeric substrates in order to inhibit the bacteria attachment [2-3]. In dental application Antonio et al. [4] reported the titanium dental implant with nitrogen physical vapor deposition (PVD) surface modification demonstrates antibacterial ability. Hence, this study aims to investigate the antibacterial ability of titanium orthopaedic implant with nitrogen plasma immersion ion implantation (PIII) surface treatment.

Methodology: Polished titanium alloys (Ti-6Al-4V) discs with 5 mm in diameter and 1mm in thickness were prepared for PIII. The Ti samples were then implanted by nitrogen plasma at various voltages of 20, 30 & 40kV at 60, 100 & 200Hz for 2 hours. The elemental depth profiles of the untreated and plasma modified samples were determined by X-ray photoelectron spectroscopy. The surface topography and roughness were determined by atomic force microscopy. To examine the adhesion of bacteria, counting colony forming unit (CFU) with the use of *S. aureus* was carried out. Medium with about $1X10^8$/ml CFU of overnight *S. aureus* culture were applied onto the surfaces. They were then incubated in 37°C with supplement of 5%CO_2 for 1 hour. Afterwards, sample surfaces were rinsed with 1ml PBS for three times in order to remove unattached bacteria. The attached bacteria were detached by adding 1ml 0.01M PBS containing 0.01% Tween 80 and then sonicated for 1 minute. The number of detached bacteria was determined by surface plating on Brain Heart Infusion agar and then incubated at 37°C for 24hrs. Bacteria morphology was visualized by LIVE/DEAD Bacterial Staining Kit (Invitrogen) and fluorescent microscope equipped with a digital camera.

Results: The depth profiles reveal nitrogen has been successfully implanted to each sample. Titanium nitride layer with higher nitrogen concentration is obtained by higher voltage and frequency implantation. The nitrogen-rich Ti layers range from 19.88nm to 99.4nm implanted by 20, 30 and 40kV, respectively (data not shown). Higher implantation voltage and frequency result to higher root-mean-square (RMS) value in which it represents rougher surface. The RMS of the control is 193Å, whereas the RMS values of N-PIII samples range from 228Å to 293Å. Therefore, surface roughness increases by PIII treatment. In CFU counting, the numbers of attached bacteria of various N-PIII samples are, however, higher than the control (Fig. 1). The surface morphologies of the control and modified sample after staining have been shown at Fig. 2a and b. Red color represents dead bacteria and green color indicates live one. Modified surface demonstrates relatively higher concentration of live bacteria than the control. These results are consistent with that of CFU counting.

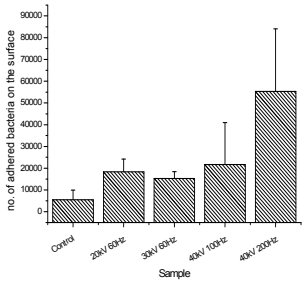

Fig.1 Number of bacteria count of nitrogen modified samples and control.

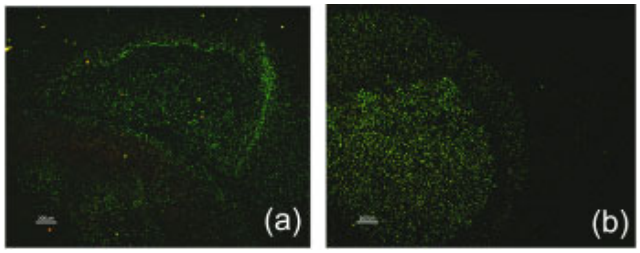

Fig.2 Fluorescent microscopic view of (a) control (b) NPIII sample with 20kV 60Hz after 1 hour bacterial culture.

Discussion:

As compared with the antibacterial function in dentistry [4], our findings confirm nitrogen plasma treated titanium implant surfaces do not suppress *S. aureus* adhesion and proliferation in which this bacterium is commonly found in orthopaedic infection. The nitrogen modified surface can only inhibit the attachment of oral bacteria and salivary proteins. It should be noted that bacterial strain in this study differs from that of in dentistry. In addition, the change of roughness and topography may promote bacteria adhesion and growth as shown in Fig. 1. Therefore, smooth surface may help suppress the attachment and proliferation of bacteria. In conclusion, nitrogen plasma modified Ti surface cannot reduce *S. aureus* adhesion and proliferation, regardless of its better biocompatibility with cell.

References:

[1]Lin et al. Appl Sur Sci;225(1-4)21-8.
[2]Tian et al. Thin Solid Films 2001;390:139-144.
[3]Zhang et al. Appl Sur Sci ;253(22)8981-5.
[4]Antonio et al. J of Oral Implant vXXIX(2),2003.