Improved Biocompatibility of Stainless Steel Implant by Titanium Coating and Micro-arc Oxidation

1Lim, YW; 1Kwon, SY; 2Sun, DH; 4Kim, HE; +1Kim YS
+Catholic University of Medical College, Seoul, Korea, 2Sun Hospital, Daejeon, Korea, 3Seoul National University, Seoul, Korea
Senior author; yongsik@korea.com

INTRODUCTION
Stainless steel is one of the most frequently used biomaterials for internal fixation device because of a favorable combination of mechanical properties, corrosion resistance, cost effectiveness and easily making a manufacturing. However, Stainless steel is not used as cementless arthroplasty implants due to their low biocompatibility because the stable oxide layer cannot be formed on the surface of stainless steel. As cementless arthroplasty implants, Ti-based alloys are widely used because of their superior biocompatibility. The titanium has a disadvantage that it is difficult to make a manufacturing and the demand of titanium has been increasing due to be used in the various fields of industry, the price of titanium is more and more expensive. We thought that if the biocompatibility of stainless steel would be improved by modifying the surface of it, we will be able to make cementless arthroplasty implants with stainless steel. Therefore, we modified surface of stainless steel by titanium coating and micro-arc oxidation process. We hypothesized that these surface modified stainless steel would enhance the biocompatibility compared to machined stainless steel.

METHODS
Specimen preparation
Forty-eight stainless steel 316L discs, measuring 12 mm in diameter and 10 mm in thickness, were manufactured. Three different type of specimens were made: machined stainless steel, surface modified stainless steel with titanium coating and micro-arc oxidation and surface modified stainless steel with grit blasting, titanium coating and micro-arc oxidation.

Grit Blasting
All substrates were cleaned ultrasonically. Grit blasting was achieved with Al2O3 particles with a diameter of 200-500 µm, moving in a high-velocity air stream (KSSA SF3, Kum-Kang tech, Korea). The roughness of grit-blasted specimens were in the range of 5 ~ 7 µm.

Electron beam Deposition
As a target material, commercially-pure grade Ti plates with dimensions of 10 mm × 10 mm × 1 mm were prepared. The discs formed substrates were mounted on the simple rotating and planetary holder inside vacuum chamber, respectively. A titanium layer was then coated on the substrates up to 5 µm at a rate of 0.1nm/s.

Micro-arc Oxidation
After the Ti coating, the specimens were oxidized electrochemically by the micro-arc oxidation (MAO) process. As an electrolyte, a solution of 0.15M calcium acetate monohydrate (Aldrich, USA) and 0.02M glycine phosphate calcium salt (Sigma, USA) was used. The applied voltage, frequency and duty of the pulsed DC power were 230 V, 660 Hz and 60 %, respectively. All of the MAO treatments were carried out in a water-cooled glass bath using a stainless steel plate as a counter electrode for 3 min.

Characterization
The surface morphologies, composition, and phases of titanium coated stainless steel were studied by means of SEM, EDS, and XRD. Biological properties of coating layers were evaluated by in vitro cell (osteoblast cell lines, Saos-2) tests, such as cell attachment, proliferation and differentiation of cell lines to confirm the improvement of their biocompatibility using Promega proliferation assay, alkaline phosphatase activity. The analysis of gene expression for osteocalcin and collagen I was done through RT-PCR. Statistical analysis was performed using the SPSS 11.5 software, with statistical significance determined as p<0.05. This experiment was repeated at least three times with similar results, according to Student’s t-test.

RESULTS
Changes in surface morphology of each step were observed with the scanning electron microscopy. The surface of stainless steel 316L substrate had sharp machining grooves (Fig. 1A). When Ti was coated on this stainless steel 316L, there are many small particles newly formed on the surface. Traces of machining groove are still visible (Fig. 1B). When the stainless steel 316L substrate coated with Ti was micro-arc oxidation processed, the surface morphology was changed markedly. All the machining grooves and the spherical particles disappeared. Instead, the surface became rough and porous as clearly. The size of pores is about 1 µm and the roughness was about 6.5 µm (Fig. 1C). Fig. 1D is the SEM back-scattered image of cross section of MAO treated specimen. It seemed there are three regions; metallic substrate region, Ti layer and porous TiO2 region above it.

Phases of the specimens were identified by X-ray diffraction (XRD) patterns. The XRD pattern of the as-machined stainless steel 316L substrate is only one peak corresponding to the stainless steel 316L. When Ti with a thickness of 5 µm was coated on the substrate by the E-beam deposition, strong Ti peaks were detected and the peak of stainless steel 316L almost disappeared. When the Ti coated specimen was micro-arc oxidation processed at 230V, peaks of TiO2 were detected in addition to those of Ti.

Biological characteristics were evaluated by in vitro cell tests, such as cell proliferation, and differentiation. The degree of proliferation of cells was determined by Promega proliferation assay of specimens. The proliferation of the cells on the Ti-coated specimen after 96 hours showed a statistically significant improvement compared to that on the stainless steel 316L substrate (p = 0.026). The ALP activity of the cells on the Ti-coated specimen after 14 days showed a statistically significant improvement compared to that on the stainless steel 316L substrate (p = 0.044). The molecular biologic analysis using the primer pair of osteocalcin and collagen I, the gene expression of osteocalcin and collagen I was observed in two groups; the increased pattern was indicated in Ti coated and micro-arc oxidation processed group but the result of quantitative analysis was not statistically significant.

DISCUSSION
The purpose of this study was to improve the biocompatibility of stainless steel materials by forming a stable porous oxide layer on the surface. Stainless steel is an attractive implant materials in terms of mechanical properties, easily manufacturing and economical points of view. However, their low biological characteristics hinder their wider applications as cementless arthroplasty implants. Titanium alloy is well known to be one of the most biocompatible materials among metals. Therefore, coated with Ti is a simple method to improve the biocompatibility of stainless steel while maintaining its mechanical properties. Actually, when Ti was coated on the stainless steel surface, the ALP activity of the cells increased markedly. Ti was coated on stainless steel so dense and uniformly that the interface was not distinguishable and bond strength was very high.

The biocompatibility of stainless steel was improved by changing surface chemical composition and characteristics. It is of note that this method is applicable to any other metals that were not able to be used as implant materials because of their low biocompatibility despite they have other advantages, such as mechanical strength, economical merits and other physical properties.

Fig. 1 SEM image of surface of (A) as-machined, (B) Ti-coated (5 µm) and (C) MAO treated (230V) specimen. (D) SEM cross-sectional image of MAO treated specimen (a) stainless steel 316L, (b) Ti layer and (c) TiO2 layer.