Enhanced biocompatibility of Co-Cr alloy by impregnating VEGF in nano-porous coating layer

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Introduction: Co-Cr alloys have excellent mechanical properties, which makes them suitable for highly loaded applications such as knee joints or vascular stents [1, 2]. However, they have relatively poor biocompatibility compared to other biomaterials such as Ti or its alloys. Biocompatibility of the Co-Cr alloys has been enhanced significantly through the formation of porous TiO_2 layer on the surface [3]. In this study, the biocompatibility of the Co-Cr alloy was improved further through the impregnation of growth factors in the TiO_2 nano-pores formed on the surface through the Ti coating followed by the ATO treatment.

Methods: Pure Ti (grade 2) was coated onto polished Co-Cr alloy specimen (10mm×10mm ×2mm) with the thickness of 2 µm by e-beam deposition. ATO treatment of Ti-coated specimen was carried out in an ethylene glycol based electrolyte containing 0.25 wt% ammonium fluoride and 2 vol% distilled water by applying a DC field with 60 V for 30 min. The change of surface morphology after ATO treatment was investigated by field-emission scanning electron microscopy (FE-SEM) images. The degree of protein impregnation was observed by a confocal laser scanning microscopy (CLSM) after impregnating green fluorescent protein (GFP) in the nano-porous coating layer. Vascular endothelial growth factor (VEGF) was impregnated into the coating layer and its effect on the biocompatibility was evaluated by *in vitro* cellular tests using human umbilical vein endothelial cells (HUVECs).

Results: Figure 1 shows the FE-SEM images of (A) as-machined, (B) Ti-coated, (C) ATO treated Co-Cr alloy and (D) fractured cross-section of the coating layer. After Ti coating, sharp machining grooves of the as-machined alloy surface became smooth, as shown in Figures 1(A) and (B). ATO treated specimen showed uniform nano-porous structure with an average pore diameter of about 100 nm and a thickness of 1 µm, as shown in Figure 1(C, D). Figure 2 shows the CLSM images of GFP impregnated (A) as-machined, (B) Ti-coated and (C) ATO treated Co-Cr substrates. Compared with as-machined and Ti-coated substrates, ATO treated substrate showed much brighter green images which means the amount of impregnated GFP. Figure 3 shows the FE-SEM images of HUVECs cultured for 2 days on the VEGF impregnated (A) as-machined, (B) Ti-coated and (C) ATO treated Co-Cr substrates. After Ti coating, the change in cell morphology was not significant. However, when ATO treated, monolayer of HUVECs was formed, as shown in Figure 3(C).

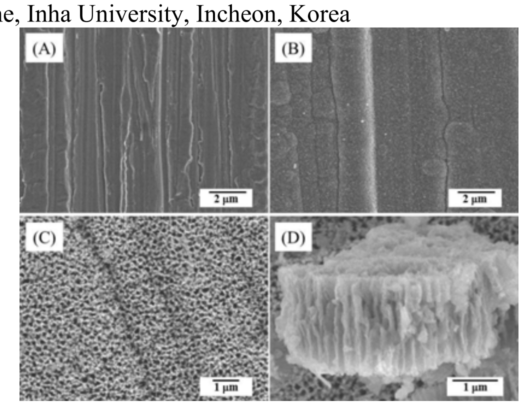

Figure 1. SEM images of morphologies of (A) as-machined, (B) Ti-coated and (C) ATO treated Co-Cr alloy and (D) fractured cross-section of the coating layer.

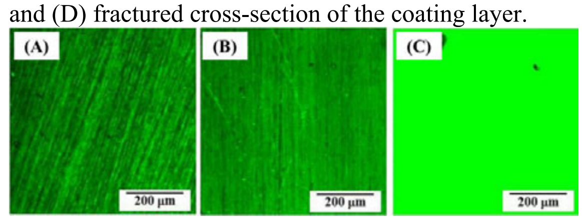

Figure 2. CLSM images of GFP impregnated (A) as-machined, (B) Ti-coated and (C) ATO treated Co-Cr alloy.

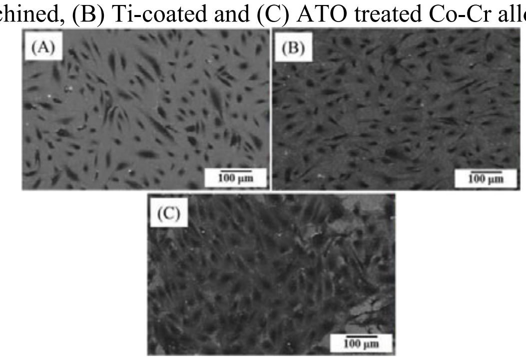

Figure 3. FE-SEM images of HUVECs on the VEGF impregnated (A) as-machined, (B) Ti-coated and (C) ATO treated Co-Cr alloy.

Conclusions: Surface modified Co-Cr alloy implant which has nano-porous TiO_2 layer was fabricated by Ti-coating and ATO treatment. After surface treatment, much more GFP was impregnated on the surface layer. Furthermore, when the VEGF was impregnated, HUVECs adhered more actively on the ATO treated surface.

References:
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