Biomimetic Incorporation of Fibronectin with Thin Calcium Phosphate Film on CP Titanium

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Statement of Purpose: Commercially pure titanium (Ti) and titanium alloy are preferred for dental and orthopedic implants or prosthesis because of their corrosion resistance, biocompatibility, durability, and strength. However, being bioinert, the fixation of such implants in bone was not in good condition. The fixation of titanium and titanium alloy implants is related to their surface composition and topography. Osteoconductive calcium phosphate layer is commonly coated on the surface of titanium implants using various coating methods for fast fixation, such as plasma spraying, ion-beam sputter deposition, pulsed laser deposition, and electron-beam evaporation with and without ion beam assist. It's no doubt that the addition of certain biologically active protein with biomaterial will improve the bioactivity of the material. Previously, we examined the biocompatibility of basic fibroblast growth factor (bFGF) incorporated with titanium (Li Y. Biomaterials 2008:29:2025-2032). In this study, we investigated the effect of fibronectin (FN) incorporated with thin calcium phosphate film deposited on titanium by electron-beam evaporation since fibronectin is actively involved in cell adhesion, spreading, wound healing, cytoskeletal reorganization, and bone tissue formation.

Methods: The biomimetic coating process was carried by immersing thin calcium phosphate film coated Ti in Dulbecco's Phosphate buffer saline (DPBS) containing FN (20 μg/ml) at 25 °C for 3 h, 6 h, and 24 h. And the same solutions but without immersing a sample were prepared for 3h, 6 h, and 24 h as controls. The surfaces of samples were examined with FESEM, X-ray photoelectron spectroscopy (XPS). The quantity of FN taken up was monitored by BCA method. The MG-63 (ATCC) cells cultured on the samples were used to evaluate the cell properties of FN-apatite composite layers, such as cell adhesion and alkaline phosphatase (ALP) activity.

Results: The amount of FN incorporated at 3 h, 6 h, and 24 h was 2.681 μg, 3.594 μg, and 5.096 μg on average, respectively. After incubation in DPBS solution, apatite was formed on calcium phosphate coated surface, and the flake-like crystals were bigger for the prolonged incubation. In comparison with apatite formed without FN, the crystal plates in the FN-apatite coating were curved, thinner, and smaller. The XPS spectra of the surface of coated Ti before and after immersion in DPBS solutions containing FN showed that nitrogen, which existed only in FN among the reagents used in the present study, was detected after immersion, and the intensity became higher with time. The number of adherent cells attached to the FN-apatite sample was nearly 30% higher than that the sample without FN after 2-hr culture. The ALP activity of seeded MG-63 was significantly higher on the FN-apatite formed titanium sample than on the only apatite formed one after 6 days culture as shown in Figure 1.

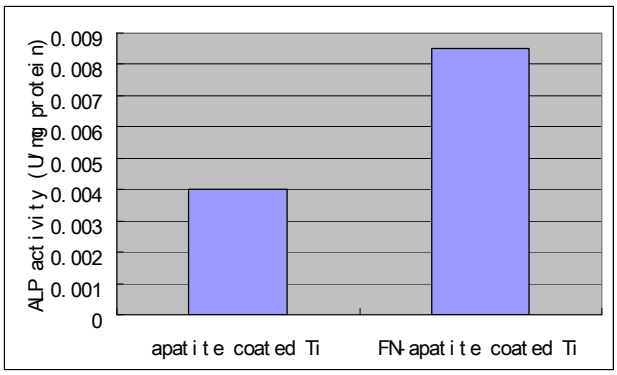

Fig.1. Incorporation FN with calcium phosphate on titanium enhanced ALP activity in osteoblasts. MG-63 cells were seeded on apatite formed only and FN-apatite formed titanium sample.

Conclusions: Considering the protein quantitative assay based on the BCA method, FESEM observations and XPS spectra, it is confirmed that a thin new apatite layer incorporated with fibronectin was formed on the calcium phosphate coated Ti. And the amount of fibronectin incorporated increased with incubation time. The incorporation of fibronectin significantly modified the morphology and crystallinity of the calcium phosphate coating. The MG-63 cell properties of fibronectin-apatite layer on titanium were superior. It is expected from the above results that titanium implant coated with a fibronectin-apatite composite will exhibit excellent bone fixation.

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