E-beam Deposited Calcium Phosphate Coating Layer as an Active Surface to Induce Bioactive Molecule-apatite Composite

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Statement of Purpose: An ideal dental implantology approach would incorporate osteocoductivity and osteoinductivity into the design of the supporting biomaterials. The bone-like apatite on titanium (Ti) enhances bone healing and apposition, leading to the rapid biological fixation of implants. Bone-like apatite coatings can also serve as carriers for biological activity molecule. An important advantage to biomimetic coprecipitation is the ability to form bone-like apatite coatings at a physiological temperature, and the conditions that the bioactive factors could release with the degradation of bone-like apatite. In the present study, thin calcium phosphate coating layer with an excellent bonding strength were deposited on titanium and its alloy by ion-beam-assisted deposition (IBAD) method. We examined the effects of bioactive molecules (BM), such as fibronectin (FN), osteogenic growth peptide (OGP) and Nell-1 protein (Nel-like molecule-1), incorporated on apatite nucleation and growth.

Methods: Calcium phosphate (CaP) film with the thickness of around 500 nm was deposited on the substrates by electron beam evaporation. Heat treatments after the deposition were performed at three different temperatures of 350 °C (sample M350), 430 °C (sample M430) and 500°C (sample M500) to get different degree in crystallinity of CaP coating layer. The biomimetic coating process was carried by immersing CaP coated Ti in Dulbecco's Phosphate buffer saline (DPBS) containing FN, OGP, and Nell-1 at 25 °C for determined intervals. And the same solutions but without immersing a sample were prepared as controls. The surfaces of samples were examined with FESEM, X-ray diffraction (XRD) X-ray photoelectron spectroscopy (XPS). The amount of BMs incorporated was monitored by bicinchoninic acid (BCA) method. The calcium ions (Ca^{2+}) concentration in solutions was measured using Calcium Assay Kit (DICA-500). OGP spatial distribution on apatite was confirmed through fluorescence. Osteoblast-like cells cultured on the samples were used to evaluate the cell properties of BM-apatite composite layers.

Results: For sample M350, Ca^{2+} concentration increased to the maximum value of 48.2 μg/ml after 15 minutes of immersion, after which Ca^{2+} concentration began to decrease. For samples of M430 and M500, Ca^{2+} concentration continuously decreased after reaching the maximum value for 1-hour immersion. The amount of BM incorporated on each sample increased with incubation time. The incorporation of BM in the newly formed layer was confirmed on the M350 samples by the presence of a nitrogen (existed only in FN among the reagents used in this study) peak in the XPS spectra. The intensity of nitrogen peaks increased with incubation time which corresponded with the results of protein quantitative assay based on the BCA method. The presence of OGP within the apatite was verified by the fluorescence, and the OGP-apatite composite was an even distribution of fluorescence. The morphology of apatite with bioactive molecules grew from small, thin, curved units to straight, flake-like, sharp-edged units for prolonged incubation, whereas, the crystal units of apatite incorporated with BM were small, thin, curved without changing crystal type even after 24 h incubation. During all the determined intervals of incubation, the amount of Ca^{2+} deposited was significantly higher on the M350 samples immersed in DPBS than on M350 samples soaked in DPBS with BM. FN, OGP, and Nell-1 protein successfully incorporated with newly formed apatite layer, and retained their biological activity to promote osteoblast-like cells adhesion, proliferation and/or differentiation *in vitro*.

Conclusions: Bone like apatite layers were formed easily when incubated in DPBS at 37 °C, and formed at a faster rate on calcium phosphate coating layer with the lowest degree of crystallinity after 15 min immersion by reaching the maximum concentration of Ca^{2+}. The maximum concentration of Ca^{2+} decreased and the time to reach it increased with the crystallinity of coating layer. The presence of BMs slowed down the rate of apatite formation, and also influenced the morphology of apatite. The BMs successfully incorporated with newly formed apatite layer, and retained their biological activity to promote osteoblast-like cells adhesion, proliferation and/or differentiation *in vitro*.

Acknowledgements: This work was supported by a grant (code #: 2009K000435) from Center for Nanostructured Materials Technology under 21st Century Frontier R&D Program of the Ministry of Education, Science and Technology, Korea