Improvement of Osteointegration of Titanium Implant by Incorporation of PTH into Biomimetic CaP Coating

Xiaohua Yu[1], L. Wang[2], X. Jiang[2], D. Rowe[2], M.Wei[1*]
[1] Department of Chemical, Materials, and Biomolecular Engineering, University of Connecticut, Storrs, CT 06269
[2] Department of Reconstructive Sciences, School of Dental Medicine, University of Connecticut Health Center, Farmington, CT 06030, USA

Statement of Purpose: Biomimetic calcium phosphate (CaP) coatings have been used extensively to enhance the osteointegration of implants to host bone by simply soaking an implant into a simulated body fluid (SBF) to grow a bone-like mineral layer. Biomolecules, such as growth factors, have been incorporated into CaP coatings to improve the osteoinductivity of the implants as CaP minerals can form chemical bonds with a variety of biological molecules. Parathyroid hormone (PTH) secreted by parathyroid gland, is a principal regulator for calcium metabolism in human body. Localized, controlled delivery of PTH can eliminate adverse effects caused by systemic administration, and maintain PTH at a therapeutic level in a long-term. The PTH-CaP coating prepared by the biomimetic coating technique combines advantages of both CaP coating and PTH, and therefore exhibits excellent osteoconductivity and osteoinductivity. The objective of this study is to develop PTH-incorporated CaP coatings for controlled release of PTH. We investigated the incorporation of PTH into CaP coating and its release behavior from the coating *in vitro,* and subsequently studied the effect of the liberation of PTH on the performance of osteoblastic cells. Implants with different doses of PTH were inserted into tibiae of mice and evaluated by a series of techniques.

Methods: Alkaline-treated titanium strips were soaked in modified SBF with different amount of PTH at 42°C for 24 h to form the PTH-CaP coating. The PTH amount incorporated into the CaP coatings was measured by enzyme-linked immunosorbent assay (ELISA). The coatings obtained were then characterized by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and field emission scanning electron microscopy (FESEM). The release profile of PTH from CaP coatings was studied by soaking PTH-CaP coatings in PBS for up to 2 weeks and the PTH release amount was determined at different time points. The effect of released PTH on osteoblasts behavior was investigated by studying the attachment, proliferation, differentiation and extracellular matrix deposition of MC3T3-E1 cells on PTH-CaP coatings. We also conducted in vivo studies to assess osteointegration of the PTH loaded CaP coating with surrounding bone in a mouse tibia model. The effect of PTH on peri-implant bone formation was examined using radiography, micro-computer tomography (micro-CT), histology, and back scattered scanning electron microscopy.

Results: It was found that PTH was successfully incorporated into biomimetic CaP coatings on titanium surface with high incorporation efficiency. The incorporation of PTH into CaP coatings had significantly changed the coating morphology, but the composition of the coating remained unchanged. Localized release of PTH occurred in vitro accompanied with partial dissolution of CaP coatings. Cell culture study demonstrated that the PTH released from CaP coatings fully retained its bioactivity. It improved substantially MC3T3-E1 cell proliferation but slightly delayed the expression of alkaline phosphatase (ALP) of the cells. Improved osteointegration of the implants loaded with PTH was observed compared to CaP coating only (the control) after 28 days of implantation in mouse tibiae. Micro-CT analysis showed better bone integration around the implant incorporated with PTH. Bone area (BA) and bone contact (BC) evaluations have demonstrated that peri-implant bone regeneration is highly dependent on the dosage of PTH incorporated. The higher the PTH content, the more bone formed surrounding the implant.

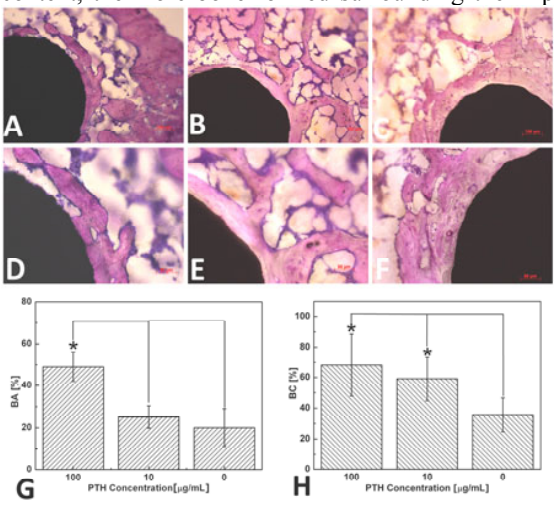

Figure 1. Histological analysis of the implants within the tibiae after 28 days of implantation: (A-C): low magnification H&E staining images of groups PTH-0, PTH-10 and PTH-100, respectively; (D-F): High magnification H&E staining images of groups PTH-0, PTH-10 and PTH-100, respectively; (G) Histomorphometric analysis of bone area (BA) 28 days after implantation; (H) Histomorphometric analysis of bone contact (BC) 28 days after implantation.

Conclusions: PTH-incorporated biomimetic CaP coatings were successfully prepared and evaluated in this study. High incorporation efficiency was obtained by adjusting the amount of PTH added to the m-SBF solution. Localized, controlled release of PTH was achieved from CaP coating both *in vitro and in vivo*. PTH liberated from the coating remained its bioactivity, which actively regulated osteoblast proliferation and differentiation. We also found PTH-incorporated-CaP coatings significantly improved the osteointegration of titanium implants by increasing bone-to-implant contact and bone regeneration.