Surface Modification of Titanium Alloy with Dextran and BMP-2 for Inhibition of Bacterial Adhesion and Enhancement of Osteoblast Function

Wilson Wang¹, Zhilong Shi², Chye Khoon Poh¹, Koon Gee Neoh²
¹Orthopaedic Surgery, National University of Singapore, Singapore, Singapore; ²Chemical & Biomolecular Engineering, National University of Singapore, Singapore, Singapore

wwej60@yahoo.co.uk

Introduction: Two key factors in joint implant failure are poor implant bonding to bone tissue and implant infection. One promising strategy to promote implant longevity is to develop bio-interactive surfaces that can enhance osseointegration while countering bacterial infection. Titanium alloy is used extensively in orthopaedic implants. Though biocompatible, its bioactivity may not be sufficient to ensure full osseointegration, and it can also fall prey to bacterial adhesion and biofilm formation, resulting in the serious complication of implant infection.

New research directions to address these issues include surface modifications of biomaterials, such as attachment of bioactive molecules like cell adhesive peptides and extracellular matrix proteins. Most reports however have looked at either host cell or bacterial interactions separately. Recognizing the importance of both, our group has worked on strategies to address osseointegration and antibacterial activity simultaneously on one modified surface.

In this report we describe a novel approach of modifying the surface of titanium alloy (TiAlV) to confer both antibacterial and osteoblast enhancing properties. Ti alloy was covalently grafted with dextran, a biocompatible polysaccharide known to inhibit bacterial cell adhesion. The resulting surface was further modified by covalent attachment of bone morphogenetic protein (BMP)-2, which has potent osteoinductive activity known to enhance bone formation in vivo. Immobilization of biomolecules on surfaces enables localization and retention of these molecules at the bone-biomaterial interface and limits uncontrolled systemic effects.

Bacterial adhesion was evaluated using S. aureus and S. epidermidis, which are common orthopaedic pathogens. Osteoblast attachment, spreading, alkaline phosphatase (ALP) activity and mineralization were investigated for both unmodified and functionalized substrates.

Materials and Methods: Dextran was covalently attached by reductive amination to the surface of 1cm² TiAlV foils using dopamine as a molecular anchor. The resulting Ti-Dex substrate was further modified by covalently attaching BMP-2 via another reductive amination process, resulting in Ti-Dex-BMP substrate. Chemical compositions were confirmed by XPS and ELISA.

For bacterial adhesion assays 10⁶ bacteria were added to each substrate in wells. Adherent bacteria after 6h incubation were dislodged and quantified by serial dilutions. Viable bacterial counts/cm² were obtained using spread plate method.

For osteoblast adhesion, 50000 cells/cm² mouse osteoblast MC3T3-E1 cells were seeded onto the substrates. After 6h incubation, adherent cells were detached by trypsinization and quantified with a hemacytometer. Cell spreading was imaged by immunofluorescence and confocal laser scanning microscopy. For ALP assay as an indicator of osteoblast activity, cells were seeded onto substrates at 50000 cells/cm² and harvested at 1, 2, and 3 week intervals. Aliquots of cell lysis solution were collected for analysis of ALP activity (Sigma). Mineralization was assayed by the amount of calcium deposited by osteoblasts after 3 weeks. Supernatants from 0.5M acetic acid dissolution were assayed for calcium content by the o-cresolphthalein complexone method.

Experiments were performed in triplicate and assessed statistically using ANOVA post-hoc Tukey test (p<0.05).

Results: Bacterial adhesion: Figure 1. Significant reductions in with Ti-Dex and Ti-Dex-BMP. (* over bar = significant difference from control)

Osteoblast adhesion: Reduced 5-fold with Ti-Dex vs TiAlV. With Ti-Dex-BMP there was no significant difference from TiAlV, indicating that BMP-2 had compensated for the anti-adhesive effect of dextran.

Osteoblast spreading: Immunofluorescence and confocal microscopy showed increased % area coverage with Ti-Dex-BMP but reduction with Ti-Dex.

ALP assay: Figure 2. Dextran consistently suppressed. Addition of BMP-s boosted ALP markedly in weeks 1 & 2. At week 3 Ti-Dex-BMP still showed a mean increase vs control, but not statistically significant.

Mineralization: Figure 3. Significant suppression by dextran was more than outweighed by the stimulatory effect of BMP-2 conjugation.

Discussion: Conjugation of dextran alone was effective in reducing bacterial adhesion, but its negative effects on osteoblast function are likely to interfere with osseointegration. However the attachment of BMP-2 in addition to dextran did not appear to interfere significantly with the desired antibacterial effect of dextran, while more than compensating for enhanced osteoblast function. This combined approach to biomolecular conjugation targeting complementary functions may have potential in future strategies to improve implant longevity.