Enhancement of Titanium Alloy with Surface-Grafted Chitosan and Immobilized RGD Peptide: Effects on Bacterial Adhesion and Osteoblast Function

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Introduction: Biomaterials are widely used in current orthopaedic practice, e.g. titanium alloy in arthroplasty. However two major impediments to their long-term success are still encountered: biomaterial-associated infections and lack of osseointegration.

Infection of orthopaedic implants is a major clinical problem with high morbidity and treatment costs. Initial adhesion of bacteria to biomaterial surfaces is believed to be an important event in the pathogenesis of infection. Subsequent bacterial growth results in biofilm formation that is resistant to host defense mechanisms and antibiotics. One approach to prevent or reduce biofilm formation on surfaces is to modify the surface chemistry such that bacterial adhesion is minimized and/or the bacteria are killed upon contact with the surface.

On the other hand, mammalian cell adhesion and proliferation is vital for successful integration of biomaterials within host tissue. Biomimetic surface functionalization through immobilization of biomolecules on biomaterials is gaining interest as an approach to modify surfaces for controlling cell and tissue responses. Adhesive peptides containing the Arg-Gly-Asp (RGD) sequence have been the focus of attention, and have been shown to enhance the growth and differentiation of osteoblasts and other cell lines.

In this study, we sought to address both these issues by exploring a novel method to enhance the surface of a commonly used orthopaedic biomaterial, titanium alloy (TiAlV), by covalently attaching chitosan (CS) [poly(1,4)-β-D-glucopyranosamine], a natural biopolymer with known antibacterial activity and lack of toxicity to mammalian cells [1]. The resulting surface was further modified with RGD peptide to enhance cell interactions. The modified materials were evaluated for activity against bacterial adhesion using S. aureus and S. epidermidis, bacteria commonly associated with orthopaedic implant infections, and also for effects on mammalian osteoblast attachment, proliferation and alkaline phosphatase (ALP) activity.

Materials and Methods: CS was covalently attached to the surface of 1x1 cm TiAlV foils using dopamine as a molecular anchor and glutaraldehyde as coupling agent. The resultant CS-TiAlV substrate was further modified by covalently attaching RGD peptide to the free terminal amine of CS, resulting in RGD-CS-TiAlV substrate. Chemical compositions were confirmed by X-ray photoelectron spectroscopy (XPS).

Bacterial adhesion assays were conducted with S aureus and S epidermidis grown in yeast-dextrose broth. 10⁶ bacterial cells were added to each substrate in a 24-well plate. Quantification of adherent bacteria was carried out after 4 h of incubation, by serial dilutions of dislodged adherent bacteria. Viable counts were estimated using the spread plate method and expressed as number of bacteria/cm². Scanning electron microscopy of adherent bacteria on surfaces was also performed.

For mammalian cell assays, mouse osteoblast MC3T3-E1 cells were used. Cell attachment, substrates were seeded with 50000 cells/cm². After 4h of incubation, adherent cells were detached by trypsinization, spun down and quantified with a hemacytometer. For proliferation, cells were seeded onto substrates at 5000 cells/cm², incubated for 1, 4 and 7 days and then harvested and quantified at the respective time points. For ALP assay as an indicator of osteoblast activity, cells were seeded onto substrates at 50000 cells/cm² and harvested after 14 days. Cell lysis buffer was added and aliquots of cell lysis solution were collected for analysis of ALP activity (Sigma kit).

Experiments were performed in triplicate. Results were assessed statistically using ANOVA post-hoc Tukey test, p<0.05.

Results: Bacterial adhesion: Figure 1.

Discussion: With our technique, the results show that the desired properties of both chitosan and RGD peptide were preserved after attachment on TiAlV, i.e. the grafted chitosan served to inhibit bacterial adhesion while the attached RGD peptide enhanced mammalian cell attachment, proliferation and ALP activity. The ability to achieve these two important functions simultaneously would be highly advantageous for implant survival and longevity by combating biofilm-related infections and promoting tissue integration.


Paper No. 62 • 6th Combined Meeting of the Orthopaedic Research Societies