Chitosan Immobilized Titanium Surface in Co-Culture System

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Statement of Purpose: Implant infection is one of the most severe complications within the field of orthopedic surgery, associated with an enormous burden for the healthcare system. Although implant materials have a high rate of success, implant-associated infection have emerged as a leading failure mechanism. Resolving such an infection usually requires multiple revision surgeries, severe discomfort for patients and an enormous problem for the healthcare system. The purpose of our research is to evaluate the osseointegration capabilities and anti-infective potential of three modified titanium surfaces in an osteoblast-bacteria co-culture system. Our previous research has used two surface modifying techniques, sulfuric acid treatment and chitosan immobilization, to address the implant-related infection and poor osseointegration (Ghimire N. Colloids Surf B: Biointerfaces 2014;122:126-33.). The development of the co-culture system simulates real-world causes of infection, such as wounds in military combat. Osteoblast cells and Staphylococcus aureus, bacteria commonly found in implant-related infections, were used in the co-culture system. Our research determines the potential of an antimicrobial, chitosan immobilized titanium surface to be used for implants to combat implant infections.

Methods: Titanium samples used in this study were prepared as described: untreated titanium (UN-Ti), sulfuric-acid treated titanium (SA-Ti), and a sulfuric acid-chitosan treated titanium (SA-CS-Ti). The Ti foil was cut into 1x1 cm^2 and ultrasonically cleaned. Staphylococcus aureus (S. aureus; ATCC) were cultured in tryptic soy broth (TSB) for 18 hours. Osteoblast-like cells, SaOS-2 (ATCC), were incubated at 37˚C at 5% CO_2 in culture media of DMEM/High glucose containing 1% non-essential amino acids, 1% antibiotics, and fetal bovine serum. Co-Culture procedure was established. Titanium samples (UN-Ti, SA-Ti, and CS-SA-Ti) were placed into previously labeled wells of 24-well non-tissue culture plates. Suspended osteoblast cells (100,000/sample) and bacteria (100,000/sample) were seeded to their respective wells simultaneously and suspended in a modified medium (98% DMEM w/10% FBS and 2% Tryptic Soy Broth). The co-cultures were incubated at 37˚C in 5% CO_2 for time points determined (30 minutes-to study initial attachment, 4 hours-to study steady-state adhesion). At each time point, modified medium was removed and samples were collected for the quantitative assay and qualitative assays: scanning electron microscopy, fixed with 2.5% glutaraldehyde, and confocal microscopy, employing rhodium phalloidin to observe osteoblast cytoskeleton.

Results: Quantitative results at 30 minutes showed that osteoblast attachment was significantly higher on the SA-CS-Ti compared to both the SA-Ti and UN-Ti (Figure 1,A), and had the lowest bacteria attachment at the 30 minute (Figure 1, B) and 4 hour time points.

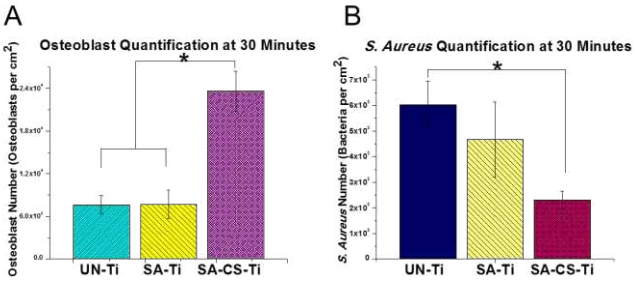

Figure 1. Co-culture graphs showing quantification of osteoblasts (A) and S. Aureus (B) at the 30 minute point. Statistical significance is denoted by * ($p<0.05$).

At 30 minutes, SEM and confocal imaging showed osteoblast attachment is rounded in morphology as the osteoblasts begin to attach. In Figure 2, SEM imaging at 4 hours show the osteoblasts spreading and displaying their polygonal morphology. S. Aureus is observed on all samples; however, bacteria are observed on the material surface of only UN-Ti and SA-Ti (Figure 2). Observations made with confocal microscopy showed normal osteoblast cytoskeletal morphology for each sample.

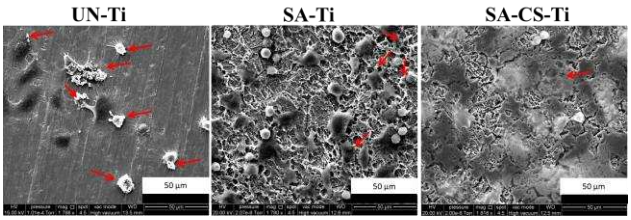

Figure 2. SEM observations of osteoblast and S. Aureus adhesion behavior in co-culture system at 4 hours. SEM micrographs of osteoblasts and S. Aureus attached on UN-Ti, SA-Ti, and SA-CS-Ti. Red arrows indicate S. Aureus.

Conclusions: In this study, we introduce the co-culture system in testing a biomaterial's efficacy in the presence of both osteoblasts and bacteria. The quantitative and qualitative results show the potential of chitosan to be incorporated into the surface of implants as osteoblast attachment increased and osteoblasts displayed normal spreading morphology on SA-CS-Ti. S. Aureus was found on the surfaces of the UN-Ti and SA-Ti, but was not observed on the material surface of the SA-CS-Ti sample showing antimicrobial influence of chitosan. This work has shown the potential for the chitosan modified titanium materials to be used in future implant design to mitigate implant related infections.

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