Osteogenic and Antimicrobial Properties of Chitosan Treated Titanium Surface in Co-Culture System of Peri-Operative Infection Model: an in vitro Study

Niranjan Ghimire [a], Yuyu Sun [b], Ying Deng [a]

[a] Biomedical Engineering Program, The University of South Dakota, Sioux Falls, SD 57107

[b] Chemistry Department, The University of Massachusetts, Lowell, MA 01854

Statement of Purpose: Not only is it the success that comes with the use of implants, but also an increased risk of bacterial associated infection (BAI). The rate of surgical site infection is 2.8 per 100 operations in USA [1]. The peri-operative route is one way of introduction of bacteria to the implant surface. The bacteria that get attached to the implant surfaces during or before the implantation procedures could colonize and form a biofilm on the implants and might result in failure of the implants. Moreover, the number of bacteria that touchdown the implants could range from as low as few hundred (in a general surgical operation) to millions (worst case scenario). We examined osteogenic and antimicrobial behaviors of chitosan-immobilized Ti in a peri-operative infection model with varying concentration of bacteria to imitate both the situations mentioned above and results for one of these concentrations are presented.

Methods: Three different surfaces were first prepared and characterized using SEM (Scanning Electron Microscope) and XPS. Commercially available untreated titanium (UN-Ti) surface was roughened with sulfuric acid (SA-Ti) and then chitosan was chemically bonded onto the surface (SA-CS-Ti).The peri-operative infection model was constructed by co-culturing the titanium substrates containing pre-attached bacteria 10^3 CFU per sample (MOI1) with osteoblasts (10^5) and the effects of bacteria to osteoblasts and vice versa on the Ti surfaces. A modified medium of DMEM, FBS and TSB (Tryptic Soy Broth) was used. After 30 minutes and 4 hours, SaOS-2 cells and bacteria (ATCC-6538) recovered from the metal substrates were quantified using hemocytometer and agar plate spreading, respectively. The cell and bacteria numbers were presented as average ± SD. The data were compared using student t-test and considered to be significant if $P<0.05$.

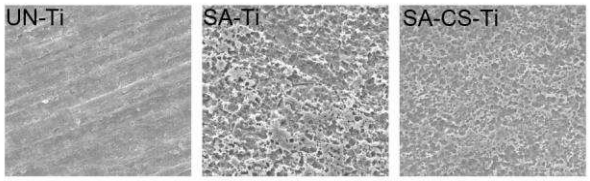

Figure 1: SEM micrographs showing different Ti surfaces

Results: Figure 1 shows topographies of different Ti surfaces. XPS showed significantly higher Nitrogen percentage on SA-CS-Ti than the other two groups (data not shown). We chose the bacteria concentration of 10^3 CFU per sample to closely mimic the microbiological environment in a general surgical procedure. While quantifying the osteoblast-like cells on different surfaces, in the infection model after 30 minutes of co-culture, the number of cells found to be significantly lower on SA-Ti samples from MOI1 than on SA-Ti from a single culture (fig.2). In addition, the cell number on the SA-CS-Ti did not show any significant change in the cell number between single culture and MOI1 (fig. 2). Moreover, higher number of cells were recovered from the UN-Ti samples in co-culture than those in a single culture (fig. 2). However, after co-culture for 4 hours, there was no significant difference between the number of cells attached on SA-CS-Ti samples with and without pre-seeded bacteria. It means pre-attached bacteria minimally affected the osteoblasts attachment behavior, and possibly does not affect the osseointegration process in the future.

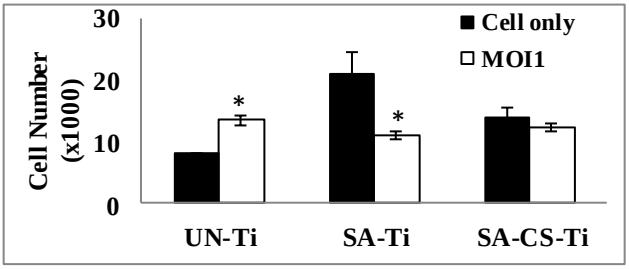

Figure 2: SaOS-2 cells at single co-cultures (for 30 min). * shows a significant difference of number between single and co-culture at $P<0.05$.

At 30-minute co-culture for the MOI1, the presence of cells significantly resulted into a lower number of bacteria attached to UN-Ti and SA-CS-Ti surfaces while there was no such effect on SA-Ti surface (table 1). Moreover, after 4 hours of co-culture, the percentage increase in the number of bacteria was found to be lower for the chitosan immobilized surface for MOI1 used in comparison to the single culture (data not shown).

Table 1: CFU count recovered from Ti- surfaces after single and co-culture for 30 min of MOI1.

	Single-culture	**Co-culture**
UN-Ti	30±20	4±2 *
SA-Ti	60±10	30±26
SA-CS-Ti	40±20	8±7*

* shows a significant difference of number between single and co-culture at $P<0.05$.

Conclusions: Here**, a** peri-operative infection model was used to evaluate the antimicrobial and osteogenic properties of Ti surfaces. Based on both the bacteria and osteoblast-like cell attachment results, SA-CS-Ti surfaces showed better antimicrobial property with competitive osteogenic activities amongst all the Ti surfaces used.

Acknowledgements: Research reported in this publication was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under Award Number R21AR065625.

References: [1] James DW. Infect Control Hosp Epidemiol., 2002; 23;183-189.