TITANIUM DEBRIS INHIBITS OSTEOBLAST ADHESION TO FIBRONECTIN SUBSTRATES IN VITRO

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Introduction: Wear debris generation has been implicated as one of the primary causes of periprosthetic osteolysis and implant loosening in total joint replacement. Metallic, ceramic, and polymeric debris particles have been shown to provoke a biologic response in tissues surrounding joint tissues. The fact that the cells immediately surrounding the implant consist primarily of osteoblasts and mesenchymal cells combined with reports of transport of the particles along the interface between the prosthesis and bone has led us to investigate the influence particulate debris has on osteoblasts *in vitro*. Titanium particulate debris has been shown to increase proliferation of fibroblasts *in vitro* and to be phagocytosed by macrophages and osteoclasts. However, there are few studies that address the functional responses of osteoblastic cells to metallic debris material, even though osteoblasts are one of the principle cell types of the tissue adjacent to the prosthesis, maintaining the physiologic bone remodeling through the balanced coordination of bone formation and resorption in concert with osteoclasts. Osteoblasts have been shown to phagocytose polymeric particles [1], however, no significant toxic effects were noted. Early responses of osteoblasts to titanium particles have not been well characterized, especially in terms of the effect particles may have on the osteoblast. To date, the phenomenon of osteoblast phagocytosis of titanium particles has been suggested, but has not been sufficiently confirmed in the literature. This study sought to clarify the influence of titanium particles on osteoblast adhesion to a matrix protein substrate after incubation with titanium particles for 0 to 72 hours, as well as confirm the occurrence of osteoblast phagocytosis of titanium particles *in vitro*, using the single cell adhesion study by micropipette micromanipulation system.

Materials and Methods: Cell Culture and Particle Exposure Neonatal rat calvarial osteoblasts were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS, at 37 C and 5% CO_2. At approximately 80% confluency, osteoblasts were seeded into six-well culture plates (Costar) at a density of 500,000 cells per well. Commercially pure (Grade 4) titanium particles (-325 mesh, Aldrich Chemicals, Milwaukee, WI) were autoclaved at 135 °C for 15 minutes prior to addition to DMEM in sterile containers to form a stock solution of 1.0% by weight of particles and medium. The titanium-medium stock solution was ultrasonicated for 30 minutes prior to addition to cell culture to minimize particulate aggregation. After four hours of incubation at 37 °C to allow for initial adhesion to the culture surface, sufficient 1.0 wt% solution of titanium particles was added to culture medium to generate a final concentration of 0.1 wt% of titanium particles in each well. Control wells were also incubated for four hours at 37 °C but did not receive any titanium particles. Particle incubation times were considered to start after addition of particles to the cultures. The specimens measured immediately after exposure to particles and 15 minutes of incubation in the micropipette chamber are considered to be measured at time = 0 hours since they received no pre-exposure to particles. Control wells (without particles) were incubated for identical times as the respective particle-exposed wells. Fibronectin-coated glass coverslips were used as the adhesive substrates for micropipette adhesion study chambers prepared as described elsewhere [2]. Osteoblasts suspended in complete medium were added to the micropipette chamber and allowed to incubate and interact with the fibronectin coating for at least 15 minutes at room temperature before adhesion measurements. Adhesion Force Measurements As described by Sung, *et al.* [3], glass micropipettes with an internal tip radius of 3.5 to 5.0 μm were filled with complete medium and mounted to a hydraulic micromanipulator (Narishige, Tokyo, Japan) with the wide end of the pipette connected to a hydrostatic pressure regulating system. The micropipette was manipulated to aspirate a small portion of the cell attached to the fibronectin substrate by using a small aspiration pressure. The force (product of the aspiration pressure and the cross-sectional area of the pipette tip) required to separate the osteoblast from the fibronectin coated substrate was measured by stepwise increases in aspiration pressure followed by retraction of the pipette with the micromanipulator. The minimum aspiration pressure required to remove the cell from the substrate was used to calculate the adhesion force. Experiments were performed at room temperature, and adhesion data were collected every 15 minutes for up to 1 hour following the initial 15 minute seeding time. The adhesion force of osteoblasts was measured at 0.5 hours, 1.5 hours, 4 hours, 24 hours, and 72 hours of exposure to titanium particles. At each time period, the particles and osteoblasts were allowed to settle in the micropipette chamber for up to one hour and the adhesion force of a group of cells was measured every 15 minutes. Statistical Analysis Comparisons between each consecutive experimental groups (between the 15-30 min. and the 31-45 min. group, and between the 31-45 min. and the 46-60 min. group, *etc.*) were performed using single-factor ANOVA. F tests ($\alpha = 0.05$, $F > F_{crit}$) were performed between experimental groups (Ti particle *vs.* control) for the adhesion force assays.

Results:

By 30 minutes, osteoblasts were observed to interact with the titanium particles and phagocytosis was confirmed after one hour of exposure by manipulating the particle-challenged osteoblasts with the micropipette. Particles remained inside and freely migrated within the cytoplasm as the cell was being aspirated into the micropipette tip (Figure 1) which shows a time-lapse view of the aspiration process of a single osteoblast. By 72 hours of incubation, cell adhesion force was significantly higher for control specimens relative to those exposed to particles for all seeding times from 0 to 60 minutes (Figure 2). Specimens exposed to particles for 72 hours exhibited reduced adhesion relative to osteoblasts subjected to 4 and 24 hours of particle exposure. At all incubation times, control specimens showed a time-dependent increase in adhesion force with exposure to fibronectin coated substrates. On the other hand, for all particle incubation times, the particle exposed group showed a relatively flat response in terms of adhesion to fibronectin with no observable fibronectin exposure time dependence, even though cells were still viable and functional.

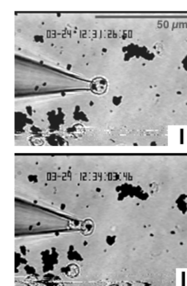

Figure 1

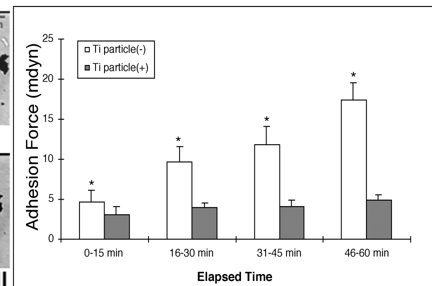

Figure 2

Conclusions: This study shows that titanium debris has a strong inhibitory effect on osteoblast adhesion to matrix protein substrates. It is also one of the first works to provide evidence for the phagocytosis of titanium particles by osteoblast cells *in vitro* (Figure 1). The effects on osteoblasts observed in this study could have implications for the role of implant wear particles on osteoblast function at the site of debris generation since osseointegration of the total joint prosthesis could be inhibited by the presence of high concentrations of titanium wear particles through a breakdown of the normal physiological balance of bone turnover. Certainly, further studies, including an *in vivo* model, will be required to fully elucidate the role that chronic exposure to titanium particulate has on osteoblast function and bone turnover. Most importantly, the results provided by this study may provide an alternative explanation for the pathogenesis of peri-prosthetic osteolysis secondary to implant wear debris generation, and may also have implications for the role of the osteoblast in the presence of phagocytosable particulate debris.

References:

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[2] Sung, K-L. P., *et al.*, *J Orthop Res*, **14** *pp.* 729-735, 1996
[3] Sung, K-L. P., *et al.*, *Proc Natl Acad Sci*, **93** *pp.* 9182-9187, 1996

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