MECHANICAL LOADING ALTERS THE SECRETION OF CYTOKINES AND PROTEASES IN HUMAN FIBROBLASTS WHEN STIMULATED BY PARTICULATE DEBRIS

+*Ninomiya, J T; *Tran, C; *Struve, J A; *Stelloh, C T
+*Department of Orthopaedic Surgery, Medical College of Wisconsin, Milwaukee, WI. 9200 West Wisconsin Avenue, Milwaukee, WI 53226, (414) 805-7430, Fax: (414) 804-7499, jninomi@ibm.net

Introduction: Fibroblasts play an important role in the development of osteolysis and implant loosening, and are seen in abundance in osteolytic membranes surrounding loose implants. They phagocytose particulate wear debris and respond by increasing the levels of cytokines and proteases, ultimately resulting in bone loss and implant failure. While it has been shown that mechanical loading may serve as a pump for the distribution of particulate debris, little is known about the interactions between mechanical loading and cellular responses in the presence of particulate debris. Therefore, we chose to investigate the effects of mechanical loading on the expression and secretion of proteases and cytokines in response to stimulus by particulate debris in human fibroblasts.

Materials and Methods: Human neonatal fibroblasts were grown to confluence in DMEM, 10% fetal bovine serum, and antibiotics, and were downshifted with serum containing, 0.5% FBS 24 hours before experiment. Following addition of particles, the cells were incubated for 72 h. Mechanical loading was carried out at 5000u strains at 1hz for 1800cycles every 24hrs on a commercially available Flexercell strain unit with 25mm loading stations. These strains represent forces within the physiologic range normally seen in bone. Following incubation and loading, supernatants were collected and frozen at – 70 C.

Titanium particles (1-5:70 C) were obtained commercially (Alpha), and were cleaned using alternating washes of nitric acid and ethanol/NaOH. Endotoxin content was performed on the particles using the Limulus amoebacyte lysate assay. Size analysis and particle concentration were evaluated by scanning electron microscopy and Coulter Multisizer.

Western blot analysis for the proteases collagenase and stromelysin were performed using commercially available polyclonal antiserum following polyacrylamide gel electrophoresis. The secretion of the cytokines IL-6 and TNF alpha were determined using match paired antibody (Endogen) ELISA kits.

All experiments were performed with triplicate samples, and statistical evaluation was carried out using the analysis of variance with the Bonferroni Dunn post-hoc modification.

Results: Western blots for collagenase and stromelysin demonstrated an increase in the basal level of secretion of these proteases following mechanical loading. Addition of titanium particles increased the secretion of the proteases, but mechanical loading decreased this effect. (Figure 1)

Unlike the proteases, the basal secretion of IL-6 was diminished by mechanical loading. Addition of titanium particles increased IL-6 levels at 0.00034 vol%, and this effect was decreased with mechanical loading. (Figure 2)

Discussion: Our study demonstrate that mechanical loading can alter the biologic effects of particulate debris. First, mechanical loading at physiological strains increased the secretion of collagenase and stromelysin in human fibroblasts, suggesting that weight bearing may increase levels of these proteases, resulting in increased bone turnover.

Secondly, mechanical loading altered the effects of particulate debris on the secretion of collagenase and stromelysin. In the absence of loading, titanium particles enhanced the secretion of the proteases, and mechanical loading diminished the magnitude of these effects.

Finally, loading decreased the secretion of IL-6, both basally as well as with the addition of particles.

While it has previously been reported that mechanical loading provides a pump-like mechanism that enhances the distribution of particulate debris, our data suggest that mechanical loading also modulates the process of osteolysis at the cellular level. Taken together, these findings may provide important information regarding the formation of osteolytic membranes and the process of implant loosening.

References: