EFFECTS OF HYDROXYAPATITE PARTICULATE DEBRIS ON THE EXPRESSION AND SECRETION OF CYTOKINES AND PROTEOLYTIC ENZYMES IN HUMAN FIBROBLASTS

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INTRODUCTION: Hydroxyapatite is a widely used calcium phosphate biomaterial that is currently used to coat total joint implants in order to obtain rapid and enhanced ingrowth into bone. Despite its documented osteoconductive effects, little is known about the fate of the inevitable particulate debris that are generated through its use. A pathologic process, Milwaukee Shoulder, is also produced by hydroxyapatite crystals, and is characterized by a florid inflammatory response leading to severe arthritis. We hypothesized that hydroxyapatite (HA) and hydroxyapatite/∃-tricalcium phosphate (HA/TCP) particulate debris might also yield similar effects, leading to the production of a variety of cytokines and proteolytic enzymes that are associated with osteolysis and bone resorption.

Therefore, we chose to assess the effects of HA and HA/TCP particles on human fibroblasts, cells that are seen in abundance in osteolytic membranes following revision surgery for loose total joint implants. We compared the effects of these materials with the results obtained using titanium and cobalt chromium particles.

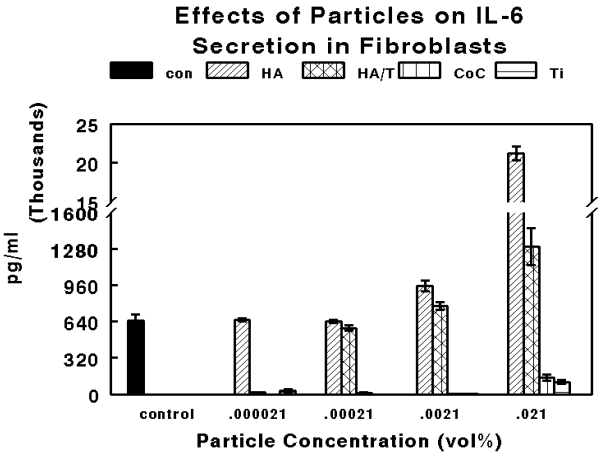
METHODS: Hydroxyapatite and hydroxyapatite/∃-tricalcium phosphate crystals were prepared by solution precipitation from calcium nitrate and ammonium phosphate and were sintered at 1100EC. Chemical composition was verified by x-ray crystallography. Titanium particles were obtained commercially, and cobalt chromium samples were prepared by cryomilling. Size analysis and particle concentrations were determined by Coulter Multisizer.

To remove any residual endotoxin, each of the samples was depyrogenated by either heating for 12 h at 200EC, or by treating for five alternating cycles in nitric acid and ethanolic sodium hydroxide. Endotoxin levels were determined on the particles directly using the Limulus amoebacyte lysate assay.

Cellular proliferation was assessed using a commercially available assay (MTS), while specific ELISA were used for the cytokines interleukin-6, inteukin-1, and tumor necrosis factor-∀ (TNF-∀). Collagenase and stromelysin secretion were determined by Western blot analysis, and protein specific gene expression was assayed by Northern blots. Zymogram gels were used to determine functional proteolytic activity.

Samples of the particles were added to confluent cultures of human fibroblasts, and dose-response and time course determinations were performed. Controls consisted of fibroblasts without any added particles, and each experiment consisted of triplicate determinations for every data point. After incubation the culture supernatants were collected and frozen for analysis. Cellular RNA was precipitated and collected for Northern blot analysis. Statistical evaluation was performed using the analysis of variance and the Bonferroni-Dunn method for multiple comparisons. Statistical significance was assigned at the 95% level. significance

RESULTS: Size analysis demonstrated that the mean particle diameter for all of the materials was <10:. All of the samples produced increases in fibroblast proliferation, with maximum effects being seen with titanium and HA/TCP particles. Both HA and HA/TCP increased IL-6 secretion, with the HA particles producing over a 30-fold increase, while the two metals were inhibitory when compared to the unstimulated controls. (Figure 1)



Time course experiments demonstrated an increase in IL-1-∃ secretion by only titanium after two hours of incubation. All of the samples inhibited the basal secretion of TNF-∀ compared to the unstimulated controls.

All of the samples also altered the levels of collagenase and stromelysin that were secreted into the particle conditioned media, with HA and HA/TCP yielding the largest increases. Functional assessment of proteloytic activity was also increased by all of the samples on zymogram gels when compared to controls.

DISCUSSION: Our data demonstrate that both HA and HA/TCP particulate debris were capable of stimulating the expression and secretion of several cytokines and proteolytic enzymes that are associated with osteolysis and bone resorption. In general, when compared to titanium and cobalt chromium particles, the HA and HA/TCP produced larger effects, yielding greater increases in both cellular proliferation along with IL-6, collagenase and stromelysin secretion. Taken together, these findings suggest that the particulate debris resulting from HA coated total joint prostheses may be capable of producing a profound and vigorous biologic response that can ultimately lead to osteolysis and implant loosening.

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