ABSTRACT INTRODUCTION:
Particle-induced peri-prosthetic osteolysis is the major cause for orthopedic implant failure. This failure is mediated mainly by the action of osteoclasts, the principal cells responsible for bone resorption and osteolysis. Therapeutic interventions to alleviate osteolysis have been focused on understanding and targeting mechanisms of osteoclastogenesis. We previously published that the Map Kinase c-Jun N-terminal MAP Kinase (JNK), acting in cooperation with NFAT, is an important mediator of particle-induced murine osteoclastogenesis in vitro.1 Thus, the purpose of this study was to examine the functional significance of this finding, i.e. the role of JNK in PMMA particle-induced osteoclastogenesis. The current study examines the role of JNK in vivo in response to PMMA particles using murine calvaria model. Furthermore, the clinical relevance of JNK-based regulation of PMMA-induced osteoclastogenesis is evaluated in human marrow macrophages which are osteoclast progenitors.

METHODS:
Commercially available PMMA microspheres (Polysciences, Inc.) 1-10 μm diameter (6.0 μm mean, 95% <10μm) were used for all experiments. Osteoclast Assay: Bone marrow macrophage from total hip replacement (THR) patients were maintained in the presence of RANKL (30ng/ml) and M-CSF (30ng/ml) for 10-14 days and then treated with control media or experimental conditions for an additional 24 hours. Cultures were then fixed, Tarrtrate Resistant Acid Phosphatase (TRAP) stained and average osteoclast (multinucleated TRAP positive cells) counts determined. Histology: Mice calvariae were preserved in 10% buffered formalin (24h), and decalcified using 10% EDTA, pH 7.0 for 7 days. Calvariae were then dehydrated in graded alcohol, cleared through xylene, and embedded in paraffin. Paraffin blocks were sectioned longitudinally. Five-micron sections were then stained with TRAP to determine osteoclasts. Osteoclasts were counted under light microscope (using 20× objectives) at the mid sagittal suture with 0.25-mm intervals as assessed by NIH Image. Each condition was run in triplicates and all experiments performed three times. Results were compared with an unpaired t-test.

RESULTS SECTION:
Osteoclast Assay - the data indicate that PMMA mounts a strong osteoclastogenic response, as measured by elevated number of TRAP-positive osteoclasts (3-4 fold increase p<0.01). Inclusion of the JNK inhibitor SP600125 (0.5μM) significantly reduced this response by blocking PMMA-induced osteoclastogenesis (70% inhibition compared with control). Having shown that inhibition of JNK attenuates PMMA-induced osteoclastogenesis by murine and human progenitors, we then tested the effect of JNK inhibition on PMMA particlengenerated calvarial osteolysis in mice. To this end, PMMA particles (10mg) were administered over the calvaria on day 0. SP600125 (0.5mg/mouse) was directly injected on calvaria on day 0 and day 4. On day 7, calvaria was collected and subjected to histologic analysis. Massive inflammation and hypercellularity were observed in PMMA-treated calvaria. Further, elevated numbers of TRAP-positive osteoclasts and large areas of focal bone erosion were evident as assessed by NIH Image. In contrast, SP600125 significantly inhibited PMMA-induced calvarial osteolysis.

DISCUSSION:
The present study demonstrates that SP600125 JNK inhibitor abolished particle-induced osteoclastogenesis in human osteoclast precursor cells from THR patients. In addition, locally administered SP600125 effectively blocked PMMA-induced osteolysis in mice calvaria model. Thus JNK might be an attractive target for orthopedic particle-induced osteolysis.

REFERENCES:

ACKNOWLEDGMENTS:
NIH AR 54326, AR 49192, Shriners Hospital for Children, and OREF Career Development Award.