Introduction: Particulate wear debris from implants plays an important role in the periprosthetic osteolysis cascade through the cellular interaction of macrophages, osteoclasts, fibroblasts and osteoblasts[1]. In fact, there have been a great deal of in vitro studies on periprosthetic loosening primarily concerned with various cellular mediators and cellular interactions. However, the precise cellular and tissue response of osteolysis is still controversial due to a lack of knowledge of its pathogenesis even when in vivo experimental models are used to simulate the aseptic loosening process [2]. Previous studies have been shown to be considerably variable, with heterogeneous results according to the site of implantation, and the type and size of particles. Moreover, the presence of wear particles alone has recently been reported to be insufficient to initiate bone resorption. Most current procedures for inducing osteolysis in an animal model not only are complex, with several steps and special equipment required, but also do not appear to induce typical bone resorption by osteoclasts in a reasonable time period. Ideally, what is needed is an experimental model that can demonstrate a quantifiable, reliable, and distinct osteolytic response in vivo within a reasonable period of time [3]. In order to determine the mechanism of wear debris induced osteolysis, a reproducible and reliable animal model is needed in the presence of a wide variety of orthopaedic implant materials. This study addresses these issues by using a mouse calvarium model which allows for the semiquantitative histological classification with respect to particle materials. This model is useful for the research of osteolysis for the purpose of prevention and therapy. This method enables the qualitative histomorphological classification with respect to particle materials. All particles were composed of clinically-relevant materials. This model is minimally invasive and simple so that many of the variables encountered with macrophages, resorption, etc. considered by this study, UHMWPE is statistically shown to be the most influential particulate debris material in terms of induction of the osteolytic cascade.

Materials and Methods: Preparation of Particles: Ti-6Al-4V particles averaging 1 to 3 µm in diameter were prepared by Zimmer (Warsaw, IN). The commercially available PMMA powder consisted of MMA-styrene copolymer particles measuring about 1 to 10 µm in diameter. UHMWPE particles averaging < 1 to 3 µm in diameter were generated from a hip joint wear simulator system (Harris Institute, Boston, MA). All particles were suspended in sterile PBS, followed by autoclave sterilization (Ti-6Al-4V) or gamma irradiation (polymeric particles). The volumetric concentration of particle suspensions were all prepared as 0.25% v/v.

- Experimental Protocol: This protocol used 12 to 16 week-old male mice, C57/BL6 strain with mean weight of 32g. The mice were anesthetized with pentobarbital sodium. The 24 mice were divided into four groups of six: Control with sham surgery, Ti-6Al-4V, PMMA, and UHMWPE, respectively. After shaving, a longitudinal skin incision was made at the midline of the cranium. This was followed by dissection of the scalp and removal of the periosteum until the coronal, sagittal and lambdoid sutures of the calvarium were visible. 100 µl of each particle suspension was dispensed by sterile micropipette onto the calvarial surface with care taken to retain a maximum amount of particle solution on suturing. In the case of the Control (sham operated) group, the same procedure was performed, except for the addition of particle solution. 

Histological Analysis: All mice were killed with cervical dislocation 1 week postoperatively. Each calvarium tissue specimen was then bisected thorough the coronal plane, followed by fixation in 10% phosphate-buffered formalin, then decalcified in 14% EDTA, dehydrated in graded alcohols, and embedded in paraffin. Three 4 µm thick non-consecutive step sections were stained using hematoxylin-eosin and tartrate-resistant acid phosphatase (TRAP) staining (polymeric particles). The volumetric concentration of particle suspensions were all prepared as 0.25% v/v. The measurements made were of number of macrophage, TRAP staining activity, resorption perimeter and total tissue cross section area(osseous area + fibrous area). The number of macrophage was counted over high power field. For TRAP activity, colorimetric analysis techniques were applied using NIH Image in order to isolate regions of colors corresponding to positive TRAP activity, with normal bone tissue color serving as a negative control. The areas of these regions were then normalized to the total tissue area (A bone) in order to obtain a relative TRAP activity per specimen, %TA. Total bone area (%A bone) was compared with total tissue cross sectional area (A total) in order to calculate ratio of bone area to total tissue area (%A bone), with the remainder of the tissue considered to be fibrous in nature. %A bone was acquired through excluding the resorption perimeter from total fibrous tissue area. In this case, a higher %A bone will correspond to less osteolysis, and a higher %A bone will correspond to a greater degree of fibromatous response. Each result was then categorized according to the extent of osteolysis and fibromatous response to the specific particle type. Differences in mean values of variables between groups were assessed by Mann-Whitney test to make allowance for multiple comparisons. (p < 0.05, significant)

Results: Relative to the Control group(Sham), Ti-6Al-4V, PMMA, and UHMWPE exposed tissue showed remarkable evidence of an inflammatory reaction, with osteolysis, proliferation of fibrous tissue, and granuloma, resulting in disruption of the normal bony structure. Many macrophages, TRAP positive osteoclast-like cells, and fibroblasts were observed at the periosteal surface and at focal lesions in the disrupted calvarium. Specifically, UHMWPE specimens showed the greatest extent of osteolytic activity, followed by Ti-6Al-4V, with PMMA exhibiting the least amount of osteolysis, i.e., %A bone (UHMWPE) < %A bone (Ti-6Al-4V) < %A bone (PMMA) < %A bone (Sham). As to A bone, Ti-6Al-4V specimens showed the highest extent of fibromatous tissue proliferation, followed by UHMWPE, then PMMA. Relative TRAP activity (%TA) was shown to be highest for UHMWPE, followed by PMMA, then Ti-6Al-4V. Overall, by all factors(i.e. macrophages, resorption, etc.) considered by this study, UHMWPE is statistically shown to be the most influential particulate debris material in terms of induction of the osteolytic cascade.

Conclusions: Osteolysis was confirmed within a short period (less than one week) which is important for rapid screening of important factors that may be helpful for the research of osteolysis process. This method enables the qualitative histomorphological classification with respect to particle materials. All particles were composed of clinically-relevant materials. This model is minimally invasive and simple so that many of the variables encountered with macrophages, resorption, etc. considered by this study, UHMWPE is statistically shown to be the most influential particulate debris material in terms of induction of the osteolytic cascade.

References:

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