An In Vivo Model of Continuous Infusion of Ultra High Molecular Weight Polyethylene Particles

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Introduction: Wear particles from orthopaedic implants have been shown to affect both initial osseointegration and subsequent bone remodeling of total joint replacements (TJRs). Continued production and exposure to particles over time adversely affects bone formation and resorption, resulting in periprosthetic osteolysis. Current animal models of particle-induced periprosthetic osteolysis have used either a single injection of particles, which is not representative of the clinical scenario, or more advanced animal species. The goal of this study was to develop and validate a murine model of continuous particle infusion in an intramedullary environment.

Materials and Methods: UHMWPE: UHMWPE particles were isolated from serum collected from in vitro hip joint simulation tests (Hospital for Special Surgery, New York). The mean diameter of the particles was <1 μm. The Limulus Amoebocyte Lysate assay (BioWhittaker, Waldersville, MD) was performed to ensure that the particles were endotoxin free. Approximately 5 x 10^10 particles were reconstituted in mouse serum (the carrier) and loaded into each pump (0.25 μl/hour delivery rate, Durect, Cupertino, CA) for infusion.

Animals: Institutional guidelines for the care and use of laboratory animals were strictly followed. Eight-week-old, wild type C57/b16 male mice were used in this study.

Animals were divided into two groups randomly. One group of animals (N=13) had a right femoral rod and infusion of carrier solution alone without particles, and a left non-operated limb. The other group of animals (N=10) received bilateral femoral rod implants. In these 10 mice, UHMWPE particles in carrier solution were infused over a 4-week period using an infusion pump and tubing in the right femur only.

Surgical Procedure: General anesthesia was induced by 3% isoflurane in 100% Oxygen. Using sterile technique, the intercondylar notch of the distal femur was exposed through a medial parapatellar arthroscopy. A 23-gauge needle was used to manually drill through the intercondylar notch to access the medullary cavity. A 23-gauge rod, 6-mm long, was press fit into the distal femur. When indicated, the rod was connected to polyvinyl tubing and an Alzet pump implanted subcutaneously between the scapulae. After implant insertion, the quadriceps-patellar complex was repositioned and the medial quadriceps arthrotomy and dorsal incision for the pump were repaired with 5.0 Vicryl sutures. Euthanasia was carried out in a CO2 chamber 4 weeks after surgery.

Microcomputed tomography analysis: A detailed qualitative and quantitative 3-D evaluation of the femur was performed of the distal 2/5 of each femur using a Scanco Viva40 microCT scanner at 10 micrometer resolution. A fixed threshold was applied to assess mineralized bone on the grey scale images. The bone volume divided by total volume (BV/TV) was used for statistical analysis.

Alkaline Phosphatase Staining: The expression of Alkaline Phosphatase (Alk Phos) was detected by a monoclonal antibody (Cat #: AF2910, R&D systems) on decalcified femur embedded in paraffin. The positively stained area was quantified using Image Pro software. The positive staining area divided by tissue section area was used for statistical analysis.

Statistical Analysis: Paired t-test was performed to compare the left femur with the right femur for each animal group.

Results: For the first group of animals that received carrier infused on one side and had a contralateral non-operated femur, the amount of bone contained in the distal 2/5 of the femur did not differ (Figure 1a). In contrast, infusion of UHMW-PE significantly reduced the bone volume when compared to the contralateral side of the same animal in which the rod alone was implanted (Figure 1b).

Discussion: This study demonstrated that continuous infusion of UHMWPE particles over a prolonged period using the mouse femur is feasible. Furthermore, continuous infusion of UHMWPE particles was shown to significantly reduce bone volume using this model. Whether these effects are due to increased bone destruction, decreased bone formation or both in this model is the subject of ongoing research.

Particles have been previously shown to suppress the expression of AlkPhos by bone marrow derived osteoprogenitors in vitro. Interestingly, with the current animal model, the AlkPhos level was unchanged with the infusion of particles. The bone reparative process initiated by the surgical trauma, and the growth factor-containing serum used as carrier in this study may have offset the possible deleterious effects of the particles on AlkPhos. Future studies using continuous particle infusion, murine genetic knockouts and over-expressors, silencing molecules and other molecular techniques will facilitate a more comprehensive mechanistic understanding of the biological processes associated with the observed osteolysis.

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