Vitamin-E highly crosslinked UHMWPE wear particles induce less osteolysis compared to virgin UHMWPE in murine calvarial bone model

David A. Bichara, M.D.[1,2]; Erik Malchau, M.D.[1]; Nanna Hylleholt Sillesen, M.D.[1]; Selami Cakmak, M.D.[1]; Orhun K. Muratoglu, Ph.D.[1,2]
[1]Harris Orthopaedic Laboratory, Massachusetts General Hospital, Boston, MA
[2]Harvard Medical School, Boston, MA

Statement of Purpose: Historically, improving the performance of ultra-high molecular weight polyethylene (UHMWPE) arthroplasty implants has been one of the central challenges in the field of orthopaedic surgery and material science. Although highly cross-linked UHMWPE has been shown to greatly reduce wear in acetabular liners in patients,[1] periprostethic osteolysis due to wear debris from bearing surfaces is the leading cause of revision in total hip arthroplasty follow-up studies.[2] A recently introduced method to alternatively stabilize irradiated UHMWPE to reduce oxidation is to incorporate vitamin E (vitE) into UHMWPE, and *in vitro* studies suggest that wear particles containing vitE might have reduced functional biologic activity and may have reduced osteolytic potential.[3-5] However, as new formulations of more wear-resistant vitE-stabilized UHMWPEs are created, the long-term effects of these materials, that are currently implanted in patients (E1™, Biomet Inc., Warsaw, IN; e-plus™, DJO Surgical, Austin, TX; Vivacit-E™, Zimmer Inc., Warsaw, IN; ECiMa™, Corin, Tampa, FL) remain unknown. In this study we aimed to quantify the level of osteolysis caused by particles generated from vitE-diffused highly cross-linked, terminally gamma sterilized UHMWPE in a murine calvarial model.

Methods: *Particle Generation and SEM Analysis* A gamma sterilized UHMWPE tibial bearing (E1™, Biomet) containing vitE (approximate 0.8% by weight, diffused after 100 kGy irradiation) and virgin UHMWPE (100 kGy irradiation and melt-stabilized) were sent to BioE Solutions, Inc. (1167 South Euclid Ave. Oak Park, IL 60304) for particle generation. Particles were characterized according to American Society for Testing and Materials (ASTM) standard F1887-05(2010) using scanning electron microscopy (SEM) analysis. *Surgical Implantation of Particles:* All actions described below were approved by the Institutional Animal Care and Use Committee of the Massachusetts General Hospital. A total of n=36 C57BL/6 male mice age 8 weeks were used for this study. Study groups for each experiment were (i) vitE-diffused UHMWPE (n=12 mice), (ii) virgin UHMWPE (n=12 mice), and (iii) shams (n=12 mice). A 10 mm longitudinal incision was made between the ears and 3 mg of polyethylene particles were distributed in the exposed area for each mouse, leaving the periosteum intact. No particles were implanted in the sham group. The incision was closed using a monofilament suture and mice were euthanized after 10 days. Micro-CT Scanning: Each calvarium was scanned individually for approximately 50 minutes at the Center for Nanoscale Systems at Harvard University using a micro-CT (X-Tek HMX ST 225) with a set voltage of 70 kV and current of 70 µA. After scans, image files were reconstructed using VGStuido MAX software and a 3D model was created. Topographical Analysis of Osteolysis Distribution: The anatomical region of osteolysis was quantified using a top view of three-dimensional calvarial images obtained using VG StudioMax software. Each bone (interparietal, right and left parietal, right and left frontal) was blindly scored by two different individuals familiar with the particle-induced osteolysis model using the following scale: 0=No osteolysis, defined as intact bone; 1=Minimal osteolysis, affecting 1/3 or less of the bone area; 2=Moderate osteolysis, affecting at least 2/3 of the bone area; 3=Severe osteolysis, defined as completely osteolytic bone. Histological Analysis and Osteoclast Quantification: Calvaria were subjected to histological analysis to confirm osteolysis. Tissue was stained with H&E and reviewed by an institutional senior pathologist. Statistical Analysis: Data was analyzed using Cohen's kappa coefficient for concordance between raters. Values were rounded to the nearest significant integer between 0 and 1.

Results: *Particle Generation and SEM Analysis*: More than 83% of the vitE-diffused UHMWPE and more than 85% of the virgin UHMWPE particles measured less than 1 µm. There was no statistically significant difference in the size of generated particles. Representative SEM images are shown in Figure 1.

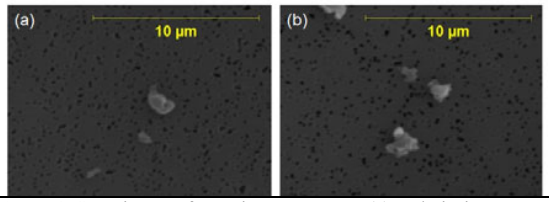

Figure 1. SEM images from vitE-UHMWPE (a) and virgin UHMWPE (b) particles.

Micro-CT scanning and topographical analysis of osteolysis distribution: Representative three dimensional micro-CT renderings of the calvaria are presented in Figure 2. The topographical analysis of osteolysis distribution revealed high inter-rater accuracy between blinded raters (Cohen's kappa > 0.70). A value of 0.94 was determined for virgin-UHMWPE (minimal osteolysis), while vitE-UHMWPE demonstrated a value of 0.35 (no osteolysis). Histological analysis: Figure 2 summarizes the histological findings. Virgin UHMWPE demonstrated a significantly greater amount of inflammatory tissue and osteolysis.

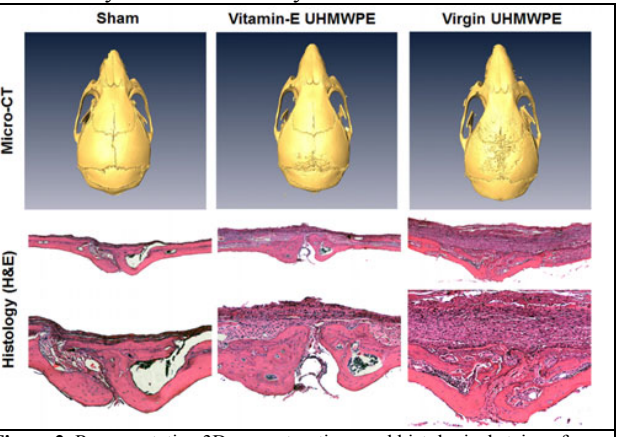

Figure 2. Representative 3D reconstructions and histological stains of calvaria after being exposed to wear debris for 10 days. Virgin UHMWPE demonstrated the greatest amount of inflammatory tissue overlying the calvaria.

Conclusions: VitE-diffused, irradiated UHMWPE particulate debris is less osteolytic and has a reduced biological activity *in vivo* when compared to irradiated and melted virgin UHMWPE particulate debris.

References: 1) Thomas, G. E. R. et al, J Bone Joint Surg Am, 93(8): 716-22, 2011. 2) Kurtz, S et al., J Bone Joint Surg Am, 89(4): 780-5, 2007. 3) Bladen, C. L. et al., Orthopaedic Research Society, Long Beach, CA, 2011. 4) Bladen, C. L. et al., J Biomed Mater Res B Appl Biomater, 2012. 5) Teramura, S. et al., Orthopaedic Research Society, Las Vegas, NV, 2009.