Particles from vitamin-E-diffused highly cross-linked UHMWPE induce less osteolysis compared to highly cross-linked virgin UHMWPE in a murine calvarial bone model

David A. Bichara, M.D., Erik Malchau, M.D., Nanna Hylleholt, M.D., Selami Cakmak, M.D., Orhun K. Muratoglu, Ph.D.
Harris Orthopaedic Laboratory, Massachusetts General Hospital, Boston, MA
omuratoglu@partners.org

Statement of Purpose: Ultra high-molecular weight polyethylene (UHMWPE) particle-induced osteolysis is one of the major causes of arthroplasty revisions [1]. Phagocytable particles can lead to the upregulation of the inflammatory cascade, resulting in bone resorption and implant loosening. Recent *in vitro* findings have suggested that UHMWPE wear particles containing vitamin-E (VE) may have reduced functional biologic activity and decreased potential to cause osteolysis. This is of significant importance since VE-stabilized cross-linked UHMWPEs were recently introduced for clinical use, and no in vivo studies have determined the immunologic response to particulate debris from VE-UHMWPE [2,3]. In this study we hypothesized that particles from VE-stabilized, radiation cross-linked UHMWPE (VE-UHMWPE) would cause reduced levels of osteolysis in a murine calvarial model when compared to virgin gamma irradiated cross-linked UHMWPE.

Methods: All actions were approved by the IACUC of the Massachusetts General Hospital. A total of n=30 C57/BL6 male mice age 8 weeks were used for this study. Study groups were the following: (i) radiation cross-linked VE-UHMWPE, (ii) radiation cross-linked virgin UHMWPE, and (iii) shams. UHMWPE particles were generated at Bioengineering Solutions, Inc. (Oak Park, IL). Particles for both materials had a similar shape and size distribution. Mice calvaria were prepped in a sterile fashion. A 10 mm incision was made on the skin using a Metzenbaum scissor. Subsequently, 3 mg of UHMWPE particles were implanted by overlaying the powder throughout the exposed calvaria. No particles were implanted in the sham group. The incision was closed using a monofilament suture and mice were euthanized after 10 days. The calvaria were harvested and processed for micro-CT scanning (Center for Nanoscale Systems, Harvard University) and histomorphometic analysis (quantification of both inflammatory fibrous tissue overlying the calvaria and osteoclasts).

Results: More than 83% of the VE-UHMWPE and more than 85% of the virgin UHMWPE particles measured less than 1 µm in mean particle size. There was a statistically significant difference between virgin and VE-UHMWPE groups (p=0.005) in regards to bone resorption. Calvaria exposed to virgin UHMWPE particles had a mean osteolytic area of 12.2% ± 8%; those exposed to VE-UHMWPE particles had a mean osteolytic area of 3% ± 1.4%. A statistically significant difference in the amount of fibrous tissue between groups (0.48 vs. 0.20, p<0.0001) was encountered; virgin UHMWPE wear particles induced the greatest amount of inflammatory tissue. No statistical significance was found between virgin and VE-UHMWPE groups (8.00 ± 6.30 vs. 3.80 ± 5.20, p=0.293) in regards to osteoclast quantification. Representative 3D micro-CT renderings and histology is presented in Figure 1.

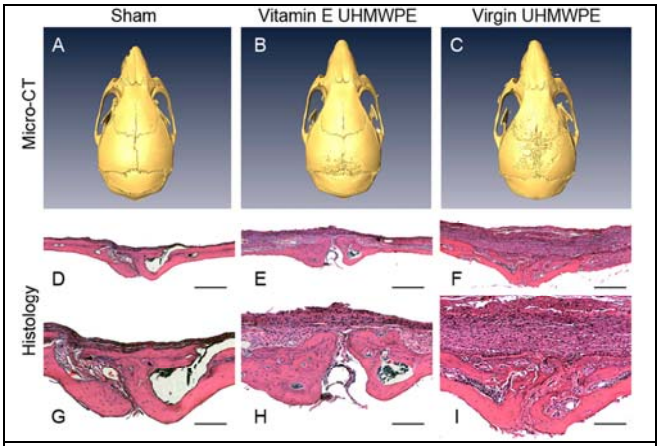

Figure 1. (A) Sham; (B). Vitamin-E highly cross-linked UHMWPE and (C). Virgin highly cross-linked UHMWPE. Histological cross sections stained with H&E (top row, low magnification; bottom row, high magnification) demonstrate the amount of inflammatory tissue caused by the UHMWPE particles. Virgin UHMWPE particles caused a significantly greater osteolytic and inflammatory process when compared to vitamin-E UHMWPE. Scale bars: Low magnification images, 500 µm. High magnification images, 100 µm.

Conclusions: The clinical use of VE-stabilized UHMWPEs has instigated various studies to determine the effects of sub-micron sized particulate debris both *in vitro* and *in vivo*. In this study we have determined that exposing murine calvarial bone to VE-UHMWPE causes less inflammation and osteolysis when compared to virgin UHMWPE after 10 days. There was no difference in the number of osteoclasts quantified between groups. This finding might be explained by the fact that osteoclasts, by day 10, have resorbed bone and migrated out of the site, leaving behind unmineralized extracellular matrix. The findings of this study are significant to the area of total joint arthroplasty. We are currently performing the same study using *in vivo* micro CT imaging to accurately quantify bone resorption and deposition.

References: [1]. Kurtz, S., et al. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. J Bone Joint Surg Am, 2007. 89(4): p. 780-5. [2]. Bladen, C. L., et al. Analysis of wear, wear particles, and reduced inflammatory potential of vitamin E ultrahigh-molecular-weight polyethylene for use in total joint replacement. J Biomed Mater Res B Appl Biomater, 101(3): 458-66, 2013. [3]. Bladen, C. L., et al. In vitro analysis of the cytotoxic and anti-inflammatory effects of antioxidant compounds used as additives in ultra high-molecular weight polyethylene in total joint replacement components. J Biomed Mater Res B Appl Biomater, 2012.