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Anti-oxidation Treatment of Ultra High Molecular Weight Polyethylene Components to Decrease Periprosthetic Osteolysis: Evaluation of Osteolytic and Osteogenic Properties of Wear Debris Particles in a Murine Calvaria Model

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Abstract Wear debris-induced osteolysis remains the greatest limitation of long-term success for total joint replacements with ultra-high molecular weight polyethylene (UHMWPE) bearings. To address oxidative degradation post-gamma irradiation, manufacturers are investigating the incorporation of antioxidants into PE resins. Similarly, larger molecular weight monomers have been developed to increase crosslinking and decrease wear debris, and ultimately osteolysis. However, the effects of modifying monomer size, crosslink density, and antioxidant incorporation on UHMWPE particle-induced osteoclastic bone resorption and coupled osteoblastic bone formation have never been tested. Here, we review the field of antioxidant-containing UHMWPE, and present an illustrative pilot study evaluating the osteolytic and osteogenic potential of wear debris generated from three chemically distinct particles (MARATHON®, XLK, and AOX™) as determined by a novel 3D micro-CT algorithm designed for the murine calvaria model. The results demonstrate an approach by which the potential osteoprotective effects of antioxidants in UHMWPE can be evaluated.

Keywords Ultra-high-molecular-weight polyethylene (UHMWPE) · Antioxidant · Wear debris · Osteolysis · Mouse model · Murine model · Treatment · Osteoarthritis

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Introduction

Total hip replacement (THR) is most commonly performed for end-stage osteoarthritis, although this procedure is also performed for rarer conditions such as rheumatoid arthritis, avascular necrosis of the hip, and femoral neck fractures. Despite its well-known propensity to generate wear debris and subsequent periprosthetic osteolysis, polyethylene bearing surfaces remain the gold standard for THR based on their consistent results and survivorship of ~85 % after 15 years [1], and the significant pitfalls of alternative articulation designs

(i.e. metal-on-metal and ceramic-on-ceramic) [2••, 3]. Thus, a major focus of ultra high molecular weight polyethylene (UHMWPE) research has been on formulations that minimize host response that leads to aseptic loosening.

One of the consequences of early UHMWPE devices, which are sterilized using high-dose gamma irradiation (25−100 kGy), while exposed to air to also crosslink the polymer chains, is the formation of free radicals that become trapped in the final product [4]. These residual reactive oxygen species (ROS), left unaddressed, cause oxidative degradation as seen in UHMWPE components stored on the shelf for a long time in air-permeable packaging prior to implantation [5], leading to increased wear and decreased performance in vivo [6]. Additionally, ROS is known to increase the host response to wear debris [7].

Subsequent improvements in UHMWPE processes introduced temperature-driven manufacturing operations, specifically remelting and annealing, which increases the molecular mobility and facilitates recombination of the free radicals. These additional processes are not without drawbacks, leading either to reduced mechanical properties or only giving a partial protection against oxidation due to ROS [8••]. While annealing methods that heat the UHMWPE to temperatures just below their peak melting point have been adopted to reduce ROS in the manufacturing process, the implants may still be sterilized with gamma irradiation after final packaging, exposing the devices to potentially significant oxidation [9•].

Yet another approach to stabilizing UHMWPE is to provide oxidation resistance without decreasing UHMWPE fatigue strength, by means of the incorporation of antioxidants such as vitamin E into the resin [10], or by diffusing it into already consolidated and radiated UHMWPE [11•]. This advance adds yet another variable to the chemical composition and molecular response to the irradiation/annealing conditions that can be used to generate novel UHMWPE implants. Since the in vivo wear debris properties of each of these constructs will need to be empirically determined to define their effects on osteoclastic bone resorption (osteolysis) and coupled bone formation (osteogenesis), there is a great need for cost-effective in vitro and in vivo models [12, 13], and the pros and cons of the current murine models have recently been reviewed [14].

Our research, aimed at understanding the biological responses to wear debris particles, has relied heavily on the murine calvaria model, which was originally developed to study titanium and polymethylmethacrylate particles with histology [15, 16]. Subsequently, this model has been used as a small animal surrogate to study novel interventions for wear debris-induced osteolysis including: bisphosphonates, cyclo-oxygenase inhibitors, TNF and RANKL biologic antagonists, NF-kappa B inhibitors, Jun kinase inhibitors, inhibitors of the NALP3 inflammasome, and adenosine receptor activators [16–26, 27••]. Most recently, this murine calvaria model was modified to study UHMWPE particles [28], using volumetric micro-CT [18]. Since osteolysis is the result of uncoupled bone resorption, an important component of the murine calvaria model is the robust bone formation that occurs on resorption surfaces within 2 weeks of wear debris implantation [29], which occurs in the complete absence of osteoclasts [22]. This allows for the analysis of both osteolysis and osteogenesis from longitudinal micro-CT data if a faithful 3D registration algorithm can be developed to quantify the wear debris-induced osteolysis (day 0 calvaria bone volume – day 10 calvaria bone volume) and osteogenesis (day 10 osteoid and under-mineralized bone volume). Here, we describe these methods in a pilot study evaluating the effects of particles from three distinct UHMWPE materials (MARATHON, XLK, and AOX), versus sham surgery and hydrogel (PVA-PAA) particle controls (Table 1), aimed at testing the hypothesis that UHMWPE particles of similar size distribution would illicit similar biological response, despite variations in the starting resin or the presence of the antioxidant.

Wear Debris Particle Generation

Particles from three different compositions of UHMWPE (MARATHON, XLK, AOX) were generated from DePuy production barstock lots using high speed cryomilling and cryopulverization (BioEngineering Solutions, Chicago, IL, USA). Particle filtering was used to isolate particles in the 1−10 um range, and they were characterized using low angle laser light scattering (Microtrac-X100,) and scanning electron microscopy with EDS to confirm particle size, shape, and composition. The major differential characteristics of these particles are summarized in Table 2. Particles were EtO sterilized and verified free of endotoxins (<0.01 uE; Kinetic QCL).

In Vivo Studies

All animal studies were performed under protocols approved by the University of Rochester Committee for Animal Resources. Eight-week-old C57B/6 mice were shaved prior to calvaria surgery, and the area was sterilized with 70 % ethanol and iodine. A 0.5–×0.5-cm area of calvarial bone was exposed by making a midline sagittal incision over the calvaria, leaving the periosteum intact. A low threshold dose of particles known to be required to induce osteolysis (2 mg) or a high dose (5 mg) of MARATHON, XLK, AOX and hydrogel (PVA-PAA) control were spread over the area of each mouse and hydrogel were directly injected onto the calvaria surface (n=6). Afterwards, the incision was closed with 2.0 interrupted sutures. One assigned group consisting of an incision of only the skin served as a sham surgery control.

Table 1 Parameters of UHMWPE composition, gamma irradiation, and annealing

Factors	MARATHON	XLK	AOX	(PVA-PAA hydrogel)
Resin (GUR)	1,050	1,020	1,020 + AO	Poly (vinyl alcohol) and Poly (acrylic acid)
Gamma dose	50 kGy	50 kGy	80 kGy	50 kGy
Remelt	Yes	Yes	No	No

Micro-CT Scanning and Osteolysis vs. Osteogenesis Analysis

Micro-CT scans were performed with a VivaCT40 (ScanCo Medical, Basserdorf, Switzerland) using an isometric resolution of 15 um. Baseline calvaria volume was obtained from in vivo scans on day 0 (before surgery), while the mice were anesthetized with 2 % isofluorine and 1 L/min oxygen. After sacrifice on day 10, the skulls were rescanned with the same parameters. The DICOM micro-CT files were then transferred to Amira v.5.4 (Visage Imaging, San Diego, CA, USA) for quantitative analysis. Quantification of the osteolytic and osteogenic volume was performed as show Fig. 1. Briefly, in vivo micro-CT scans of the calvaria are performed prior to surgical implantation of the wear debris particles (day 0), and ex vivo scans of the calvaria are performed after tissue harvest on day 10. The DICOM files are used to generate an initial 3D image of the calvaria at each time point, and these images are then imported into the Amira program for volumetric registration and analyses. Three distinct tissues types were defined by this process based on their bone mineral density (BMD). In the region of interest (ROI), the original calvarial bone, which has a high BMD (zoom and data window=1,000–7,000; display and masking=1,700–7,000), was initially identified. Then an under-mineralized tissue with a lower BMD (zoom and data window=1,000–3,000; display and masking=1,000–2,500), was identified within the ROI, which we defined as new bone that formed in response to the wear debris-induced osteolysis. Finally, the unmineralized soft tissue, with a lower BMD, was identified within the ROI between the original calvaria, and the new woven bone was defined as inflammatory tissue. Based on this tissue segmentation, we were able to calculate the volumes and determine the osteolytic versus osteogenic potential of the different particles via liner regression analysis (Fig. 1).

The results from the sham and hydrogel-treated calvaria demonstrated minimal osteolysis and the lack of a significant osteogenesis response in these groups. In contrast, all three particles displayed a significant osteolytic and osteogenic effect vs. sham controls ($p<0.01$). Additionally, all three particles demonstrated a dose-dependent effect on both osteolysis and osteogenesis, in which the effects of the 5-mg dose appeared to saturate the host responses. Therefore, lower doses are recommended for future studies. The results from the 2-mg dose produced some interesting trends that warrant further investigation. Of note was that the MARATHON particles induced the smallest osteolytic response among the UHMWPE particles tested, suggesting that at low doses it elicits the best biological response. However, when we analyzed the osteolytic versus osteogenic potential of the particles, the MARATHON particles appear to have uncoupled osteolysis and osteogenesis with a slope=0.59 (Fig. 1). Thus, this uncoupling highlights the importance of measuring both bone responses when evaluating UHMWPE particles for biocompatibility and toxicity. In contrast, AOX particles slightly favor osteogenesis over osteolysis (slope=1.27). This suggests that the presence of the antioxidant may produce a more favorable environment for bone formation following wear debris-induced bone resorption.

Conclusions and Future Directions

Under non-pathologic conditions, wear debris-induced bone resorption is coupled to bone formation to prevent osteolysis. This is why only a small fraction of joint replacement patients develop periprosthetic osteolysis and aseptic loosening, while most patients display a linear wear rate over time [30–32]. The theory that aseptic loosing may be due to an uncoupling of osteogenic/osteolytic processes rather than a specific negative

Table 2 Physical characteristics of the UHMWPE particles

	MARATHON		XLK		AOX	
	Average	Range	Average	Range	Average	Range
Particle size (μm)	2.67	0.48~23.08	3.9	0.82~24.94	3.16	0.11~17.18
Aspect ratio	1.73	1.1~4.63	1.86	1.04~8.11	1.75	1.07~16.94
Roundness	0.62	0.22~0.91	0.59	0.12~0.97	0.64	0.06~0.94
Form factor	0.64	0.12~0.92	0.57	0.14~0.88	0.58	0.01~0.91
Perimeter (μm)	11.48	1.78~138.26	17.59	2.94~15.50	14.47	0.43~194.87

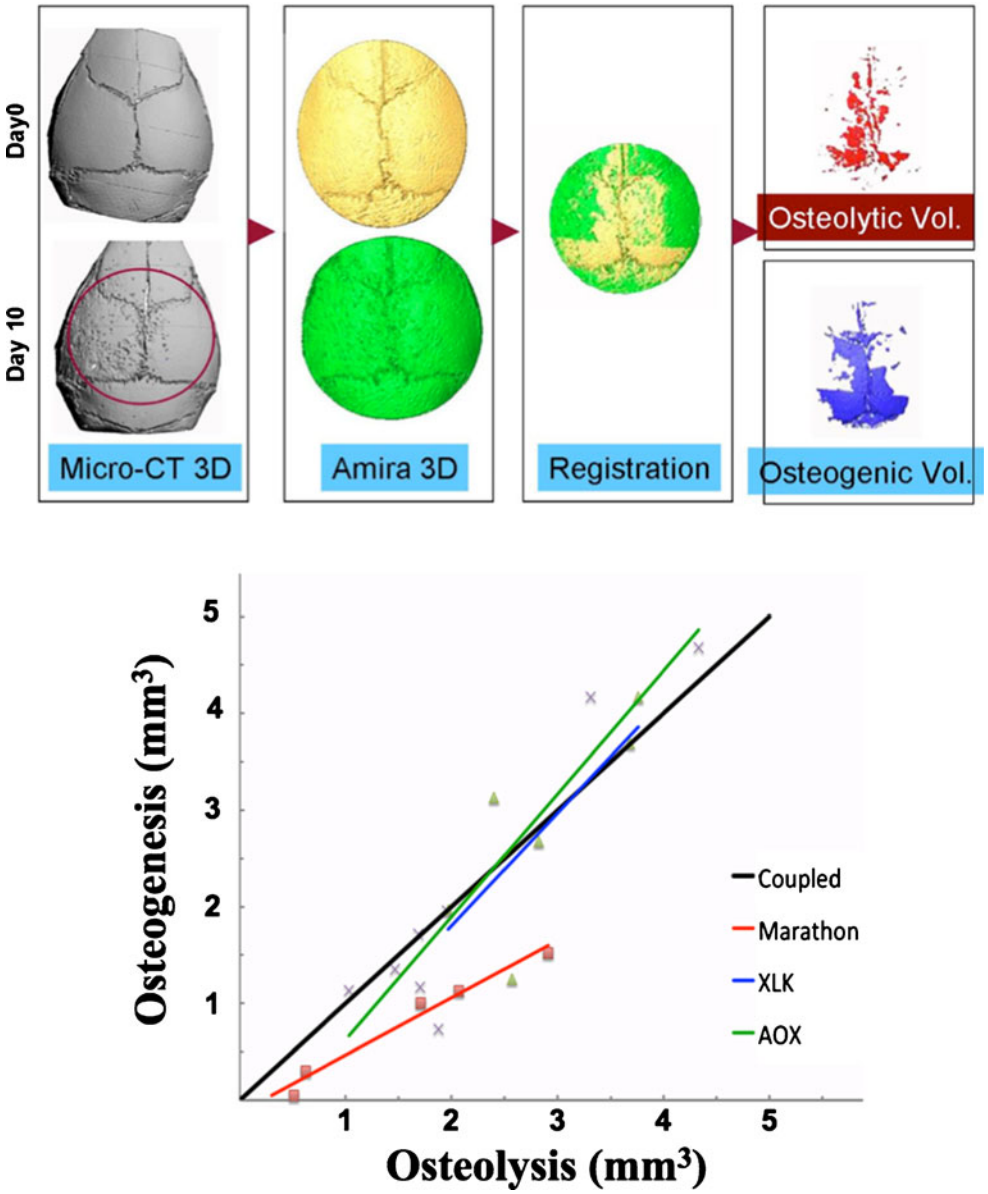

Fig. 1 Longitudinal micro-CT analysis and quantification of UHMWPE particle induced osteolysis and osteogenesis in vivo. 3D reconstructions of DICOM images were generated from the micro-CT raw data of the region of interest (ROI) (*circled* bone) obtained on day 0 and day 10 following surgical implantation of 2 mg of UHMWPE particles. After ROI confirmation via Amira image analysis, the day 0 (*yellow* bone) and day 10 (*green* bone) ROIs are co-registered in 3D. The osteolytic volume is then determined by the bone void in the day 10 ROI, and the osteogenic volume is calculated from the under calcified bone in the ROI of the co-registered 3D images. To assess the relative osteolytic versus osteogenic potential of a particular UHMWPE particle, a linear regression analysis is performed by plotting the osteolysis versus the osteogenic volume for each mouse (n=6), in which slope= 1.0 signifies perfect coupling

wear debris responses is supported by longitudinal volumetric CT analysis of patients with varying degrees of periprosthetic bone loss [31–33]. It is for this reason that we have chosen micro-CT as the primary outcome measure in our preclinical studies, and aimed to develop faithful quantitative measures of coupled vs. uncoupled responses to wear debris. Here, we demonstrate that volumetric longitudinal micro-CT can be used to quantify these events using the murine calvaria model. Using this model, we observed differences in the induced osteolysis. Material factors which may have contributed to these differences include the average molecular weight of the resin (5 vs. 2 million), the presence or absence of the antioxidant, but also the average particle size. In this experiment, attempts were made to control for the particle size, but filtering still produces a distribution of particle sizes with significant overlap, which is an issue that requires attention in the future. Moreover, inclusion of anti-oxidants into the larger particles from lower molecular weight resin without remelting results in UHMWPE (AOX) that has similar osteolytic and osteogenic properties to low dose MARATHON, suggesting a biological effect of the anti-oxidants that compensates for the lack of ROS release from remelting.

One limitation of our pilot study is that the UHMWPE particles differed in more than one variable (Tables 1 and 2). Thus, we are not able to make firm conclusions about the effects of anti-oxidant incorporation. However, it was interesting to see that the AOX particles induced less osteolysis than the XLK particles, and significantly more osteogenesis than the MARATHON ($p<0.05$). Formal studies are now planned to directly assess the potential effect of a free radical scavenging antioxidant presence on the overall osteolysis process.

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