Biological Response to Highly Cross-linked and Vitamin E-doped Polyethylene - A Wear Particle-induced Osteolysis Animal Study

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Introduction: Ultra-high molecular weight polyethylene (UHMWPE) has been used extensively in orthopedic joint replacements. Wear debris generated on the UHMWPE articular surface may induce a periprosthetic inflammatory reaction, leading to osteolysis and consequent aseptic loosening of components. Highly cross-linked polyethylene (HXLPE) is gamma irradiated to increase the bearing wear resistance. Although HXLPE offers better wear resistance in laboratory studies, some defects have been indicated during the process, such as the emergence of residual free radicals, leaving the potential for material oxidation and a decrease in mechanical properties along with lower fatigue crack propagation resistance in vivo. Thus, polyethylene containing antioxidants (vitamin E) are further developed to eliminate any residual radicals; without changing the mechanical properties of the materials. Vitamin E-stabilized HXLPE offers better mechanical and fatigue resistance properties compared to similar materials without an incorporated antioxidant. However, the biological response of periprosthetic inflammation to this polymer’s wear debris is still not well reported. This study aimed to investigate the biological response of different polyethylene wear debris in a particle-induced osteolysis animal model.

Methods: Three commercially available polymers (UHMWPE, VE-UHMWPE and HXLPE) from Orthoplastics Ltd. (Lancashire, United Kingdom) were compared. Submicron-sized wear particles were mass produced by microfabricated surface texture technology. The mean size and aspect ratio of the wear particles were measured by scanning electron microscopy to identify their size distribution. The animal study was approved by the Institutional Animal Care and Use Committee (MMH-A-S-102-1). Thirty-two 11-week-old C57BL/6 female mice (BioLASCO, Taipei, Taiwan) were randomly divided into four groups. The animals in group 1 had a skin incision only and served as a sham surgical control. The animals in group 2, 3 and 4 were treated with UHMWPE, VE-UHMWPE, and HXLPE particles respectively (n=8). Each particle-treated group was implanted with the same mass (1 mg) of wear debris. It was assumed that each particle-treated group suffered the same external stimulation. Osseous properties including the bone volume relative to the tissue volume (BV/TV), trabecular thickness (Tb. Th) and bone mineral density (BMD) were examined using micro computed tomography. For histological analysis, the sections were stained with hematoxylin/eosin (HE) and tartrate-resistant acid phosphatase (TRAP). Proliferation of soft tissue and aggregation of osteoclasts were evaluated as features of bone resorption. The data were evaluated for statistical significance by one-way analysis of variance (ANOVA) and paired Student’s two-tailed t-test. Data are reported as mean ± standard deviation (SD).
**Results:** The mean size of UHMWPE, VE-UHMWPE and HXLPE particles was 0.71±0.3 μm, 0.61±0.5 μm and 0.47±0.3 μm, respectively. The average particle size of HXLPE was significantly smaller than those of UHMWPE and VE-UHMWPE (p<0.05). Approximately 90% of the particles were granular in shape in three groups and no detectable differences were observed in aspect ratio.

The results of 3D image reconstruction of the skull surface of the mouse showed greater erosion in the particle-treated groups than in the sham group (Figure 1). The HXLPE group appeared most lacerated around the volume of interest (VOI) of the midline suture of the skull (Figure 1(d)). A decreased BMD was found in the particle-treated groups compared to the sham group. The lowest BMD was seen in the HXLPE particle-treated group, when compared with group 2 and group 3 (Figure 2(a)). The HXLPE particles produced a significant decrease in BV/TV when compared with the other groups (Figure 2(b)). This data suggested that HXLPE produces a significant decrease in Tb. Th (Figure 2(c)).

In the sections stained with HE, group 1 displayed normal skull morphology around the suture midline (Figure 3(a)). The tissues exposed to polyethylene particles showed a noticeable inflammatory reaction with soft tissue proliferation (Figure 3(b,c,d)). A more severe reaction was seen in the HXLPE group (Figure 3(d)). The implantation of PE particles caused more vigorous osteoclast aggregation compared to the sham group. The HXLPE particles induced the most violent osteoclast aggregation within the particle-treated groups.

**Discussion:** Vitamin E (α-tocopherol) has been incorporated as an antioxidant into conventional UHMWPE and highly cross-linked UHMWPE in an attempt to decrease surface delamination caused by oxidative fatigue. However, vitamin E could be diffused in-vivo or eluted with the generation of wear particles. Mice with genetic vitamin E deficiency and fed a tocopherol supplemented diet were studied to test the influence of antioxidants on bone properties. It was reported that the additional vitamin E may decrease bone density and stimulate osteoclast fusion.1 The in-vivo effect of vitamin E on particle-induced osteolysis has not been well studied. The biological mechanism for inducing osteolysis requires further study because the period of VEPE in clinical applications is too short to predict the risk of an osteolytic response. In a similar animal study, Jarrett et al.2 performed a good study for evaluating the effect of in-vivo elution of vitamin E from acetabular liners of total hip replacements using a canine model. The results demonstrate that vitamin E doped UHMWPE has no observed adverse effects on the surrounding tissues at 12 weeks follow-up. However, that study implanted a polyethylene plug containing vitamin E in a rabbit knee and a total hip replacement in a large canine; the biological responses of wear particle-induced osteolysis have not been tested in their study. The in-vivo particle-treated animal study found that the VE-UHMWPE group showed no significant difference compared to the conventional UHMWPE group. This study indicated that the possible elution of vitamin E in vivo from wear processes may not induce an adverse osteolytic response. This study could provide new insights into particle-induced osteolysis using vitamin E doped polyethylene.

**Significance:** Wear debris induced osteolysis is the main factor affecting the long-term successful rate after joint replacement. The novel vitamin E contained wear debris may not affect the inflammatory and osteolytic responses in current in vivo particle-induced osteolysis animal model. It is assumed that the morphological size and total cumulative volume of the wear debris could be the critical factors in determining the biological activities and inflammation responses. The prevalence of periprosthetic osteolysis for these commercially materials should be justified by more, longer term clinical follow-up studies.
FIGURE 1. Reconstructed image of the VOI with the midline suture of the skull. The VOI is defined with a diameter 4 mm. (a) Two weeks after sham-surgery without particle implantation (group 1). (b) UHMWPE particle implantation (group 2). (c) VE-UHMWPE particle implantation (group 3). (d) HXLPE particle implantation (group 4).

FIGURE 2. Bone resorption parameter quantified by micro-CT in calvarial tissues. (a) BMD. (b) BV/TV. (c) Tb.Th. (n=8/group, Mean ± SD, *p<0.05, **p<0.01, ***p<0.001)
FIGURE 3. Photomicrograph of HE staining in murine calvarial tissues (magnification x10, scale bars = 500 µm) (a) Two weeks after sham-surgery without particle implantation (group 1) (b) UHMWPE particle implantation (group 2) (c) VE-UHMWPE particle implantation (group 3) (d) HXLPE particle implantation (group 4).

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